SCIENTIFIC VALIDATION OF ANTI-DIABETIC, ANTI-DYSLIPIDEMIC AND ANTI-OXIDANT ACTIVITIES OF SIDDHA HERBO MINERAL FORMULATION *"LINGA MATHIRAI"* IN-VIVO AND IN-VITRO MODELS

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Scientific Validation of Anti- Diabetic, Anti-Dyslipidemic and Anti-Oxidant Activities of Siddha Herbo-mineral Formulation *"Linga Mathirai*"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.Velpandian M.D(S), Ph.D.,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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This is to certify that the dissertation entitled "**Scientific Validation of Anti- Diabetic, Anti-Dyslipidemic and Anti-Oxidant Activities of Siddha Herbo-mineral Formulation *"Linga Mathirai*"** is submitted to the Tamilnadu Dr.M.G.R.Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr.A.Shanuvas** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

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ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Scientific Validation of Anti- Diabetic, Anti-Dyslipidemic and Anti-Oxidant Activities of Siddha Herbo-mineral Formulation *"Linga Mathirai*"** is a bonafide work carried out by **Dr.A.Shanuvas** under the guidance of **Dr.V.Velpandian M.D(S), Ph.D.,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Chennai - 106.

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ABBREVIATION

AGE	Advanced Glycation End Product
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BUN	Blood Urea Nitrogen
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals.
DNA	Deoxyribonucleic Acid
DTNB	Dithionitro Bis – Benzoid acid
ESR	Erythrocyte Sedimentation Rate
FTIR	Fourier transform infra red spectrometer
GDM	Gestational Diabetes mellitus
GLC	Gas Liquid Chromatography
GOT	Glutamic Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase
GPx	Glutathione Peroxidase
GSH	Glutathione
Hb	Haemoglobin
HbA_1C	Glycosylated Haemoglobin
HDL	High Density Lipoprotein
HPTLC	High Performance Thin Layer Chromatography

IAEC	Institutional Animal Ethical Committee
IDDM	Insulin Dependent Diabetes mellitus
ICP	Inductively Coupled argon Plasma
ICPOES	Inductively Coupled Plasma Optic Emission Spectrometry
IU	International Unit
LDL	Low Density Lipoprotein
LMCV	Lymphocytic Choriomeningitis Virus
NIDDM	Non Insulin Dependent Diabetes Mellitus
NPH	Neutral Protamine Hagedorn
OECD	Organization for Economic Co-operation and Development
OES	Plasma Emission Spectroscopy
PCV	Packed Cell Volume
RBC	Red Blood Corpuscles
RNA	Ribonucleic Acid
SEM	Scanning Electron Microscope
STZ	Streptozotocin
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TGL	Triglyceride
TLC	Thin Layer Chromatography
VLDL	Very Low Density Lipoprotein
WBC	White Blood Corpuscles
WHO	World Health Organization

1. INTRODUCTION

Siddha system of medicine is an ancient indigenous medical system that has flourished on the Tamil soil over a span of more than hundreds and hundreds of years. This system of medicine is a complex of spirituality and healing technology that exploits herbal, metal, mineral substance, standing of course on tested theoretical foundations.

The traditional Tamil system of medicine should have existed from time immemorial even before the written history was made. It is very difficult to say when it was exactly originated.

Even before 2000 years the monumental Tamil composition "*Thirukural*" gives a synoptic account of the basis of medical theory and practice in ten extremely condensed two line verses.

"மிகினும் குறையினும் நோய்செய்யும் நூலோர்

வளிமுதலா எண்ணிய மூன்று."

\- திருக்குறள்

As per the above verses Saint '*Thiruvalluvar'* insisted that there are three Humours which regulates the body such as Wind, Fire and Flame, if any derangement of this three humours diseases will occur.

Siddhars were a disparate band of poet – philosophers with wide range of interests and capabilities. Their compositions are replete with philosophies of life which are unorthodox and radical in nature. Nevertheless, they are essentially men of medicine, and their philosophy is the philosophy of medicine. Their compositions comprised profound insights into securing the health of human body and mind as well. Their knowledge of the medical uses of a whole parts of herbs, metals and minerals should indeed so comprehensive and eclectic are their conceptions of medicine that they should not only make for a rewarding complement to all Synthetic drugs, but give a new perspective to our philosophy of healing.

The aim and goal of Siddhar is to attain perfection or a longevity life, were achieved through intellect or yoga.

Siddhar '*Thirumular'* is one who practiced yoga to attain perfection. *Konganavar, Chattaimuni, Karuvurar, Ramadevar* says that those who try to worship God through the inner principle will attain perfection [1].

One of the basic concepts of Siddha system is that human body (Microcosm) and the cosmos (Macrocosm) are identical. The cosmos is composed of 5 primordial elements (*Pancha bhuthas*).

Thee (Fire) manifests in *Neer* (Water), Neer manifests in *Vayu* the activated wind, which manifests in *Mann* (Earth). *Mann* and *Katru* (Wind) are interchangeable. These five primordial elements constitute the basis of everything. The cosmos is composed of their conglomeration with open mindedness, one get things materialized through the aid of these primordial elements [2a].

In this modern epoch many chronic diseases are due to lifestyle modification. One among the disease is the '*Madhu megam*' which is a great warning worldwide.

The word '*Madhu*' means honey, the word 'Megam' in the Siddha system is related to urine. Hence, passing urine with sweetness is called '*Madhumegam'*. The name Madhumegam is suitable for the symptoms of passing urine with sweetness. Siddhars have explained the 10 stages of *Madhumegam* called *Aavathai* and also the symptoms in each stage while mentioning about medicines for *Madhumegam,* they have prescribed both herbal and mineral preparations.

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbance of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion, insulin action, or both.[2b] The major symptoms of Diabetes mellitus is increased thirst and hunger, frequent urination, weight loss, fatigue, skin infection etc.,

It is explained in modern medicine that Diabetes Mellitus is caused by the ruining of beta cells in the Pancreas due to various cause by which the secretion of insulin is affected.

In Siddha system, *Kaya Kalpa* medicines which can revive the beta cells secreting insulin have been explained. This is a metabolic disorder, hence *Kaya Kalpa* medicines which can revive the ruined beta cells have been prescribed by the Siddhars long ago [3].

PREVALENCE

Diabetes mellitus currently affects more than 62 million Indians, which is more than 7.1 % of Indians adult population. An estimate shows that nearly 1 million Indians die due to Diabetes every year. The average age of onset is 42.5 years.

In 2014, India topped the world with the highest number of people with Diabetes Mellitus. The prevalence of Diabetes is predicted to double globally from 170 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2030, Diabetes mellitus may afflict upto 79.4 million individuals in India.

India currently faces an uncertain future in relation to the potential burden that Diabetes may impose upon the country. Many influences affect the prevalence of disease throughout a country and identification of those factors is necessary to facilitate change when facing health challenges. So what are the factors currently affecting Diabetes in India that are making this problem so extreme.

The aetiology of Diabetes in India is multi-factorial and it includes genetic factors coupled with environmental influences such as obesity associated with rising living standard, steady urban migration and lifestyle changes. Yet despite the incidence of Diabetes within India, there are no nationwide and few multi-centric studies conducted on the prevalence of Diabetes and its complications. The studies that have been undertaken are also prone to potential error as the heterogeneity of the India population with respect to culture, ethnicity, socio – economic condition means that the extrapolation of regional results may give inaccurate estimates for the whole country.

Rough estimation show that the prevalence of Diabetes in rural populations is one quarter that of urban population for India and other Indian sub-continent countries such as Bangladesh, Nepal, Bhutan, Sri Lanka etc [4].

Mortality and morbidity rate

Estimated 24 million persons with Diabetes in India in 2000 and this number is predicted to rise to almost 70 million people by 2025. The country with the largest number of Diabetic people will be India.

In recent study in Chennai, nearly 25% of population was unaware of a condition called Diabetes [5].

The world wide mortality of these disease Diabetes should be as follows South Africa – 91.67, Mexico – 89.56, Indonesia – 58.79, Sri Lanka – 47.87, Australia – 15.84, Malaysia – 23.86, India – 25.40, China – 14.80 and United state – 14.78 [6]. Hence, Diabetes mellitus is a life style disorder, which is a problem faced majorly by the world today.

Siddha medicine is a eccentric one, it is not only a curative, but also preventive. It helps to achieve the healthy body and also mind. Siddha medicines rejuvenate the body.

Siddhars have contributed numerous herbo mineral preparations through their literature. Siddhars obtained the products from nature. According to Siddha Materia Medica the medicines are obtained from herbs, metals, minerals and animals products.

Siddha system of medicine has numerous numbers of formulations for various diseases. Siddha medicine plays an effective role in treating Diabetes mellitus. In the use of synthetic drugs, too many adverse drug reactions like Cardiovascular disease, neuropathy, nephropathy, retinopathy, foot damage, skin changes, Alzheimer's disease etc., may occur and the long term course of drug is some time fatal.

Hence, there is a need for the new drugs formulation for these major problems, which should be less toxic, low cost and short term use. Here the drug **"*Linga Mathirai*"** is considered to satisfy the above need, and is a unique herbo mineral formulation against Diabetes mellitus.

Based on the above fact, an attempt was made to validate the Siddha herbo mineral formulations of "***Linga Mathirai"*** for its Anti-diabetic, Anti-dyslipidemic activity in animal models and Anti-oxidant activity in DPPH assay.

2. AIM AND OBJECTIVES

Aim

Diabetes mellitus is a metabolic disorder and now it is a lifestyle disorder which is a great threat to the mankind. As there is a great change in our day to day activities, food habits, physical activities etc., this disease has got its own spurt worldwide. Particularly India is the leading one as it is getting more westernized. This disease needs treatment with safe medication and quality living. This could be achieved by Siddha system of medicine as it not only treats the disease but also brings the overall wellbeing of the human.

According to the Siddha literature ***Linga Mathirai*** was used for Diabetes mellitus. Thus the aim of this study was to validate the safety and efficacy of the test drug ***Linga Mathirai*** for Anti-Diabetic Activity in Streptozotocin induced Wistar albino rats.

Objectives

The following methodology was adopted to evaluate the safety and efficacy of the test drug in this study

- Collection of various Siddha and modern literature relevant to the study.
- Preparation of the drug according to the classical Siddha literature.
- Evaluate Physico chemical, bio-chemical analysis to standardization the test drug.
- To estimate the percent of elements, functional groups and particle size through instrumental analysis of the trial drug.
- Evaluation of the Acute and 28 days repeated dose Toxicity of test drug according to OECD guidelines.
- Evaluation of pharmacological study of the drug through the following:
    - Anti-Diabetic Activity-Streptozotocin induced diabetes in Wistar albino rats.
    - Anti-Dyslipidemic Activity-Triton WR 1339 induced Dyslipidemic in Wistar albino rats.
    - Anti-Oxidant Activity-Through DPPH assay
- To scrutinize all the above studies to establish the potency of ***Linga Mathirai.***

3. REVIEW OF LITERATURE

3.1. DRUG REVIEW

3.1.1. SIDDHA ASPECT

INGREDIENTS OF *LINGA MATHIRAI*

- *Lingam (*Cinnabar)
- *Sivanar vembu (Indigofera aspalathoides)*
- *Naabi (Aconitum ferox)*
- *Nalla Ennai (*Sesame oil*)*

LINGAM [7]

Metonymy: *Inkuligam, Raasam, Kadaivanni Karppam, Kalikkam, Kaanjanam, Kaaranam, Saaniyam, Chendooram, Maniragam, Milecham, Vani* and *Vanni.*

“மணிவாரி இங்குலிகம் வன்சாதிலிங்கம்

தணிவாரும் கார்குணம் சாரும்-பிணிமாலை

தாழ்ந்த பவி குழலாய்த் தாங்காத் திரிதோடம்

வீழ்ந்தான்றும் என்றே விளம்பு...”

- அகத்தியர் [8]

“வன்னியின் கெர்ப்ப மகத்தான உண்

கன்னிய பெருமான் காரணமாம் லிங்கம்

தன்னிச்ச மரசம் சார்வான செந்தூரம்

அன்னிப் பிறந்திடும் லிங்கத்தின் பெயரே....”

- சட்டமுனி [9]

"மலைவாரி மலைராசம் மணிநாகம்" [10]

Vernacular Names

Sanskrit	-	*Lingam*
Telugu	-	*Inglieekam*
Kannada	-	*Chayilyam*
Hindi	-	*Hingool*

Properties

It is hard; it fumes when it exposed to fire; not soluble in water; has no smell and taste**.**

Suvai (Taste)	:	Tasteless
Thanmai (Nature)	:	*Veppam*

Action:- Tonic

General character

பேதிசுரஞ் சந்நி பெருவிரண நீரொடுத

காதகடி காசங் கரப்பான்புண்- ணோத

வுருவிலிங்க சங்கதமா யூறுகட்டி யும்போங்

குருவிலிங்க சங்கமத்தைக் கொள்.

ஆதி யிரதவுக் காதலாற் சாதிலிங்க

மோதி லிரதகுண முற்றூடலிற்-றீதுபுரி

குட்டங் கிரந்தி கொடுஞ்சூலை வாதமுத

லுட்டங்கு நோய்களையோட் டும்.

- குணபாடம் தாது சீவ வகுப்பு

Lingam is effective in the treatment of Diarrhoea, Pyrexia, Delirium, Urticaria, Diuresis, Tuberculosis, Scabies, Syphilis, Leprosy, Eczema, Skin diseases, Throbbing pain (*Soolai)* and *vatha* diseases.

Purification

Lingam can be purified by many methods as follows.

1. 1400 gm of Alangium bark (*Alangium salvifolium*) is powdered and added with 5.2ltrs of rice vinegar in the pot and placed it in the dew at night. The next day, it is kindled well. 35grms of *Lingam* is tied well in a cotton cloth and put it into the above liquid. The pot is covered with lid and sealed with mud pasted cloth, it is dried and exposed in the dew for one day. Then they are heated with low intensity flame until the liquid is completely evaporated. The *Lingam* is taken out and cleaned well. This procedure is repeated with the whole plant of *Vitis lanata* (*Pulikarunai*) and the root of *Hemidesmus indicus* (*Nannari*).

சொல்லக்கேள் புலத்தியனே மகனே யிந்தத்

துறையான சாதிலிங்க சுத்தி தானே

வெல்லக்கே ளழிஞ்சில் புளிங்கருணை யோடு

மேலான நன்னாரிக் காடித் தண்ணீர்

புல்லக்கே ளதிலோர் மூன்று வைகல்

புகையாமல் விளக்கிலெரி யழுக்கு நீங்கும்

வெல்லக்கே ளளவுதொடிக் கொன்றே வீசை

வெறுந் தண்ணீர் நாலுபடி வீத மாமே.

- குணபாடம் தாது சீவ வகுப்பு

2. Lime juice, cow's milk and the Indian Acalypha juice are mixed in equal proportion and allowed to process *Lingam* so as to get it in a consolidated potency state.

3. When the crude form of *Lingam* is soaked for one day in mother's milk and lemon juice respectively, it becomes purified.

4. *Lingam* is soaked in mother's milk for 30 *naazhigai (*30×24=720 mins). It is removed and again fresh milk is added and the process is repeated for two times.

Toxic symptoms of *Lingam*

- Loss of taste, difficulty in eating and drinking water.
- Ulcers in the buccal floor, Uvula (base of the mouth), inner portion of the tongue, larynx and large intestine.
- Foul odour from the mouth, discharge is viscous, whitish saliva.
- Difficult to speak and burning sensation are the toxic features of *Lingam*.

Antidote [11]

- Nutmeg (*Myristica fragrans*)
- Cubeb pepper (*Piper cubeba*)
- Root bark of red cotton tree (*Gossypium arboreum*)
- Sugar

All the above ingredients were taken equal quantity of 4.2gm are made into a decoction and administered twice a day for 48 days.

1. SIVANAR VEMBU [12a]

Other name: *Kandhari*

Vernacular name:

Malayalam - *Manaveli*

Sanskrit - *Shivanil*

Kan - *Shivamalli-gida*

Properties

Suvai (Taste) : *Kaippu*

Thanmai (Nature) : *Veppam*

Pirivu (Division) : *Kaarppu*

Action:- Antiseptic, Stimulant, Demulcent and Disinfectant.

General character

"குட்டஞ் சிரங்கு குறைப்புப் பிசமாந்தை

கட்டப் பிணிகள் கழலுமே - திட்டம்

உரனிம்பங் காயத்துக் குண்டாகு மேலை

அரனிம்ப மென்னுமருந் தால்."

- தேரையர் [13]

Uses [14a]

- The leaves, flowers and tender shoots are said to be cooling and demulcent action. They are employed as decoction in leprosy and cancer, elephantiasis and alternative in Secondary Syphilis.
- The root is chewed as a remedy for tooth ache.
- The whole plant is grinded with butter and applied to reduce oedematous tumors.
- A preparation is made from the ashes of the burnt plant to remove dandruff .
- The leaves are applied to abscesses; and oil is obtained from the root which is used to anoint the head in erysipelas.
- This is one of the important ingredients of the specific oil for syphilitic and other skin diseases.
- A decoction of the entire plant is given as an alternative in Secondary Syphilis, Psoriasis etc.

2. NAABI [12b]

Other name: Vashanabi, Vidam, Marutham.

Vernacular name

Mal	-	*Vatsanabhi*
Tel	-	*Vatsanabhi*
Assam	-	*Bish*
Hin	-	*Bachhnag*
Sans	-	*Vatsanabi*
Kan	-	*Vatsa- nabhi*

Properties

Suvai (Taste)	:	*Kaippu*
Thanmai (Nature)	:	*Veppam*
Pirivu (Division)	:	*Kaarppu*

Action:- Diaphoretic, Diuretic, Anodyne, Antipyretic, Narcotic, Sedative.

General character

"வாதவலி மந்தமறல் மாறாக் கபப்பிணிகள்

ஓதுகுட்டு குன்மந்தேள் ஓடுங்காண் - காதலர்தம்

புத்தியோ டாருயிரும் பூவும் வளைகுழலே!

சுத்திசெய்த நாவியின்பேர் சொல்."

- அகத்தியர் [15]

Purification

The root is treated with urine or milk from the cow or with cow dung for three or more days. The urine, milk or dung being renewed every day.

Uses [14b]

- Removes "*Vatha*" and "*Kapha*" alleviates inflammatory throat complaints and fevers, stimulates the secretion of bile, a general remover of internal inflammation.
- Eighteen varieties of which ten are very poisonous. Used in leprosy and inflammatory complaints of the throat and lungs.
- It is a very effective medicine in various diseases acting as a narcotic, sedative and stimulant. Useful in fever, cephalalgia, infections of the throat, dyspepsia and rheumatism.
- Internally, it is chiefly used in the treatment of chronic intermittent fevers. The drug is chiefly employed in India in the treatment of leprosy, fever, cholera and rheumatism.
- A Preparation of the root is much used in all the hilly districts in India to poison arrows. The toxic principle is the alkaloid pseudaconitine.
- Tincture of aconite at first slows the heart rate, lowers the blood pressure and increases the peripheral circulation, later the heart rate is accelerated and the blood pressure is raised.
- After treatment of the root with cow's urine the tincture increases the rate and systole of the heart, the blood pressure and the peripheral circulation, and the effects persist for a very long time (K.C. Bose, Mhaskar and Caius).
- If the root is treated with cow's milk, instead of urine, the above changes are much more pronounced (Mhaskar and Caius).
- The crude root contains about 14% of total alkaloids, whereas the root treated with cow's urine and exposure to sunlight have brought about a partial change of the toxic alkaloids aconitine and pseudaconitine into far less poisonous substances benzoyl-aconine and veratroyl-aconine (K.C. Bose).

3. NALLA ENNAI

Other name: *Thilam*

Vernacular name

Tel - *Nuvulu*

Mal - *Karuella*

Eng - Gingelly oil plant

Sans - *Tilam*

Kan - *Ellu*

Hin - *Thil*

Properties

Suvai (Taste) : *Inippu*

Thanmai (Nature) : *Veppam*

Pirivu (Division) : *Inippu*

Action:- Demulcent, Laxative, Nutritive, Emollient.

General Character

"புத்திநயனக் குளிர்ச்சி பூரிப்பு மெய்ப்புளகஞ்
சத்துவங் கந்தி தனியிளமை - மெத்தவுண்டாங்
கண்ணோய் செவிநோய் கபாலவழல் காசநோய்
புண்ணோய்போ மெண்ணெய்யாற் போற்று."

- அகத்தியர் [15b]

The seeds are acrid with a sharp bitter sweet taste, oleagenous, toxic, cooling, galactagogue, diuretic, astringent to the bowels, aphrodisiac.

Promote the growth of hair, useful in diarrhoea, gouty joint, urinary concretions, eye disease, applies to ulcers and piles cause "*Kapha*" and biliousness.

The oil from the seeds are strengthening, useful in dry cough, asthma, diseases of the lungs, burning sensation while micturation, diseases of the ear and eye, scabies, smallpox, gouty joints, syphilitic ulcers, inflammations.

In Ceylon, the oil is used for cooling the body. The seeds pounded with Jaggery are taken to purify the blood [14c].

In South Africa, natives use the seed as an aphrodisiac.

Sushruta prescribes the leaves in the treatment of snake bite and scorpion sting.

3.1.2. MODERN ASPECT OF DRUGS

1. CINNABAR [16]

Metonymy

Cinnabarite, Vermillion, Vermilion Chinna red.

Introduction

Cinnabar is the chief mineral composed of the element of mercury and is very important ore of mercury. It is a colourful mineral that adds a unique colour to the mineral colour palette. The term is also used to describe the bright red colour of this element.

Occurrence

It occurs in many parts of world, particularly in California, China, Spain, Italy &United States.

General properties

Table 1:- Physical properties Cinnabar

Colour	Bright scarlet red or brick red
Luster	Adamantine to sub metallic in darker specimens
Transparency	Crystals are translucent to transparent
Crystal system	Hexagonal
Hardness	2 to 2.5
Specific gravity	8 to 8.1
Associated minerals	Pyrite, quartz, mercury and Dolomite, calcite.

Table 2:- Chemical properties of Cinnabar

Chemical formula	HgS
Composition	Mercury (II) sulfide
	1) 86.22% - mercury (Hg)
	2) 13.78% - sulfide (S)

HgS which has long been used in combination with traditional Siddha & Chinese medicine as a Sedative, hypotonic, ant- inflammatory ,anti pyretic & analgesic for more than 2000 years and is still widely used in Asian countries.

The estimated human therapeutic dose of cinnabar in traditional medicine used to approximately 5 -25 mg /kg /day /per dose three times / day as indicated.

An overdose of cinnabar in drugs such as Bapuslsan, which is used as a sedative & for management of external intoxication in the Chinese population.

It must be aware of its toxic effects due to high mercury content. Previous studies have shown that the insoluble form of HgS (or) cinnabar can still be absorbed from GIT and liver.

Uses

- Cinnabar is to be used commonly because of its physical properties in the art work of ancient times due to its interesting red colour.
- It is used in the making of instruments; traditionally it was used to recover gold sediments or streams and in used as a fungicide.
- It is the principle ore of mercury. So the mercury is removed from this rock and used in the instruments such as thermometers and such.
- It was also used as a powder called vermilion, which in small amounts could be used as food colouring.

Medicinal Uses

- Cinnabar is grinded with lemon juice for 3hours. It is extremely efficacious drug in liver disorder such as commencing cirrhosis of liver, dyspepsia, chronic dysentery and similar other allied diseases like chronic diarrhoea.
- In secondary syphilitic eruptions, a powder composed of two parts of cinnabar is used for fumigation.
- An ointment of cinnabar is applied to bring about the resolution of buboes.
- In Meta physical ore, cinnabar has positive effects on the immune system and blood.

Toxic symptoms of Cinnabar [17]

Most of the soluble salts of mercury are absorbed slowly from the intestinal mucous membrane of the alimentary tract and produce their toxic effects.

After the absorption mercurial salts are excreted into the caecum and colon as sulphides, in this form the mercury is found in the fecal matter.

The long term use of cinnabar contained in traditional medicines could result in renal dysfunction due to accumulation of mercury in kidney.

Blurred vision due to accumulation of mercury in brain is possible.

Skin allergic reaction may occur when cinnabar is used in tattoo dyes.

BOTANICAL ASPECT

2. Indigofera aspalathoides [18]

Classification

Kingdom	:	Plant Kingdom
Class	:	Dicotyledons
Sub class	:	Polypetalae
Series	:	Calyciflorae
Order	:	Rosales
Family	:	Leguminosae
Sub family	:	Fabaceae
Genus	:	*Indigofera*
Species	:	*aspalathoides*

Distribution

Plains of Karnataka and Ceylon.

The common habitat of *Indigoferaaspalathoides* is dry sandy place. It may grow well near the coastal regions

Morphology

The plant is a sub shrub and grows erect. The young branches are characteristically purple with dense covering of minute trichomes. The leaf is

trifolliolate and sessile. The flowers are purple, solitary and axillary, the pods are straight and cylindrical with sparse trichomes, and seeds are cuboidal and smooth.

Analysis of the leaves and stems

Water	:	80.5%
Protein	:	3.1%
Fat	:	0.4%
Soluble CHO	:	6.4%
Fiber	:	2.8%
Nutritive ratio	:	3.3%
Starch equivalent	:	12.916/100

The leaves and stems constitute a source of vitamin A and C.

Phytochemical investigation has revealed the presence of many compounds that are biologically active such as salicylic acid, B-sistosterol-gluco puranoside and crythroxydiols ‘X’ and ‘Y’.

3. Aconitum ferox

Vernacular name

Kingdom	:	Plantae
Class	:	Eudicots
Order	:	Rananculales
Family	:	Rananculaceae
Genus	:	*Aconitum*
Species	:	*ferox*

Distribution

Alpine Himalaya of Nepal

Morphology

Roots biennial, paired, tuberous, daughter – tuber ovoid – oblong to ellipsoid, stem is erect, with or without a slender, hypogaceous base which emits numerous fine roots near the upper end, simple, erect [19].

Leaves scattered, distant excepting the lowest 2 or 3 which are usually decayed at the time of flowering. Inflorescence a loose raceme.

Chemical Constituents

The total alkaloid content in commercial Aconitum ferox varies from 0.63% to 4.7%. Pure roots of Aconitum ferox contain the alkaloids pseudaconitine, chasmaconitine, indaconitine and bikhaconitine, recently two new alkaloids veratroyl pseudaconitine and diacetyl pseudaconitine [20].

The use of aconite in criminal cases and for homicidal purposes is well known.

4. SESAME OIL

Sesame oil is known as the "Queen of Oils", it is obtained from the seeds of *Sesamum indicum* plant.

Sesamum is one of the most ancient of cultivated crops in India. It is the first oil seed cultivated by man. Sesamum seed has been an essential article in Hindu religious ceremonies and has been referred to as *Homodharya* and *Pitrutarpana* in ancient scripts. Sesame seeds yield on expression edible oil [21].

Constituent of Sesame oil

The Glycerides composition of the oil appears to be less effected by climatic and other factors during growth of the crop.

Fatty acid Composition

Unsaturated Fatty Acid

Mono unsaturated Fatty Acid (Oleic acid)	-	41%
Poly unsaturated Fatty Acid (Linoleic acid)	-	45 %

Saturated Fatty Acid

Palmitic Acid	-	9 %
Stearic Acid	-	5 %

Other Chemical Composition

Sesame seeds contain 50 – 60% of fatty oil which is characterized by two lignases are Sesamin and Sesamolin. Two phenolic antioxidants are gained during raffination.

Unsaponifiable matter	-	1.5 – 2.3%.
Total sterols	-	0.35 – 0.54%
Free sterols	-	0.20 – 0.24 % (β-sistosterol).
Phospholipids	-	0.034 – 0.132 %,
Lecithin	-	52%
Cephalin	-	40.6 %

δ – Tocopherol, lignins, calcium are also present.

Uses

1. Sesamol and Sesaminol
   - Maintain fats (LDL and HDL)
   - Promote integrity of body tissues.
   - Enhances Vitamin E activity to provide antioxidant capacity to the body.
   - Sesamin promotes a balanced immune and auto immune response.
   - δ – Tocopherol has little Vitamin E activity.
   - Lignins promote healthy liver.

2. Sesame oil:
   - Is a cell growth regulator and slows down cell growth and replication.
   - Is used as healing oil for thousands of years, used after exposure to wind or sun, it will calm burns.
   - Is a natural anti inflammatory agent.
   - Retains and contain calcium which is an important nutrient for entire vascular system.
   - Helps maintain normal fat level.
   - It used in the treatment of diabetes, hepatitis and migraine.
   - Used before and after radiation treatment, sesame oil helps neutralize the flood of oxygen radicals.
   - Is naturally antibacterial for common skin pathogens as Staphylococcus and Streptococcus as well as common skin fungi such as athlete's foot fungi and gingivitis.
   - Is naturally antiviral.
   - In Vitro, sesame oil inhibited replication of Human Cancer cells.
   - In both small intestine and colon, some are nourished by fat instead of sugar and the presence of sesame oil can provide the cells with essential nourishment.

3.2. DISEASE REVIEW

3.2.1. SIDDHA ASPECT OF THE DISEASE

MADHUMEGAM

Madhumegam comes under *Neerinai Perukkal Noi* as mentioned in *Therayar Maha Karisal.*

"நீரிருவினைக் குணத்தை நீயறிவிரித்துச் சொல்வாம்

நீரினைப் பெருக்கலொன்று நீரினையருக்க லொன்று

நீரிழிவுடனே கொல்லும் நீர்க்கட்டு வினைகளென்று

நீணிலமுறைக் குமிந்த நீர்நிறைக் குணத்தைக் கேளாய்."

- தேரையர் [22]

Synonym

Mega neer, Vegu moothiram, Innipu neer, Neerizhivu, Thithippu neer, Pramegham.

Definition[23]

Madhumegam is a clinical condition characterized by frequent urination resulting in deterioration and diminution of seven thathus and loss of weight.

Abdomen distends, slurring of speech, peripheral neuritis, lassitude, dyspnoea are the symptoms of *Madhumegam*.

Etiology

- ✓ Diet habits
- ✓ Sexual indulgence
- ✓ obesity
- ✓ Psychosomatic cause
- ✓ Hereditary
- ✓ Excess stimulation of *moolatharam*

General symptoms

1. Thirst
2. Polydipsia
3. Anorexia
4. Delirium
5. Sleeplessness
6. Hiccough
7. Anaemia
8. Polyuria
9. Cough
10. Dyspnoea
11. Pain in the hip and burning sensation
12. Loss of Weight
13. Flatulence
14. Giddiness

Classification of the disease

Classification of *Megam* [24]

Vali - 4

1. *Neimananeer*
2. *Pasumananeer*
3. *Seezhmananeer*
4. *Sadhaimanane*

Azhal – 6

1. *Yanaikozhupu Mananeer*
2. *Katrazhai Mananeer*
3. *Chunna Mananeer*
4. *Ennipu Megam*
5. *Palingu Neer*
6. *Muyal Kuruthi Neer*

Iyam - 10

1.Iyaneer
2.Thuimaineer
3.Moolaineer
4.Ilaneer
5.Ulneer
6.Thavalaneer
7.Kazhuneer
8.Thenneer
9.Uppuneer
10.Kavichuneer

Diabetes mellitus is a clinical entity in a modern medicine is closely resembles one of the types of "*Pitha Premeham*" i.e "*Madhumegam"*.

Clinical features

Polyuria, polyphagia, polydipsia, perspiration, exhaustion, insomnia, giddiness and loss of weight even at normal consumption of food.

Pathophysiology [25]

In the disease *Madhumegam*, due to internal and external causes affect balance in the ratio of *Vali*, *Azhal*, and *Iyam*. The imbalance affects the *Keelnokkukal*, which in turn affect the seven *Udal thathukkal*. *Saram* gets affected and there is loss of appetite. *Seiner* also get affected with the net result even if the patient eats more nourished food (Polyphagia), there won't be any improvement in health.

An imbalance in *Iyam* does imply an imbalance in other two *kutrams* too and causes derangement of *dasavayu* and seven *Udal thathukkal* which causes the disease and other complications.

3.2.2. MODERN ASPECT OF THE DISEASE

Diabetes mellitus

Diabetes mellitus is one of the most common endocrine disorders. It is a clinical syndrome characterized by hyperglycaemia with or without glycosuria, resulting from an absolute or relative deficiency of insulin, affecting carbohydrate, protein and fat metabolism. It may be due to impairment of insulin production or its release by Beta cells of islets of Langerhans. Long standing metabolic derangement is associated with functional and structural changes in many organs especially the vascular system leading to Diabetic retinopathy, neuropathy and nephropathy and atherosclerosis.

The three major types of Diabetes mellitus

- Type 1 Diabetes.
- Type 2 Diabetes.
- Gestational Diabetes mellitus

Type 1 diabetes mellitus

It is called as the insulin dependent, immune mediated or juvenile onset diabetes. People with this type of diabetes produce very little or no insulin. They need injections of insulin everyday to control the glucose level in the blood.

Type II diabetes

It is called as the non-insulin dependent diabetes. It is also known as late-onset diabetes and it is characterized by insulin resistance and relative insulin deficiency. Though the disease is highly genetic in origin some risk factors such as excess weight, inactivity, high blood pressure and poor diet plays a major role for its development.

Gestational Diabetes mellitus (GDM)

It is the presence of high glucose level in the blood during pregnancy. It usually disappears after the pregnancy but the women and her children are at high risk of developing type II diabetes later in their life.

Complications [26]

Virtually every tissue and organ is biochemically and structurally altered as a consequence of the hyperglycemia of diabetes and result in complications.

Two biochemical mechanisms appear to be involved in the development of many complications.

(i) Non enzymatic glycosylation

It is the process by which glucose attaches to amino group of proteins without the aid of enzymes. That can cause structural and functional abnormalities of the involved proteins to form Advanced Glycation End Products (AGE), which cause microangiopathy, nephropathy. The concentration of Glycosylated Hemoglobin (HbA_1C) in the blood is now used clinically as a measure of therapeutic control.

(ii) Intracellular Hyperglycaemia with disturbances in Polyol pathways

The second biochemical mechanism operates in the aorta, lens of the eye, kidney and peripheral nerves. These tissues are endowed with an enzyme, aldoses reductase that facilitates the accumulation of sorbital and fructose in cells of the hyperglycemic patient. As a result of the intracellular accumulation of sorbital and fructose an osmotic gradient is established and excessive amounts of water enter the cells from the extra cellular compartment. The cells then swell and are damaged and contribute to neuropathy and cataracts.

1. Diabetic ketoacidosis

It is a major medical emergency and serious cause of mortality principally in people with type 1DM. It has a mortality rate of 6-10%.In newly diagnosed patients of type 1 DM there is failure of endogenous insulin and in NIDDM it is due to inadequate exogenous insulin or a stressful conditions like myocardial infarction, hyperthyroidism, pheochromocytoma, trauma, pregnancy, drugs like cocaine. In these conditions there is increase in production of counter regulatory hormones like epinephrine, cortisol, glucagon and growth hormone.

The cardinal biochemical features are

1.Hyperglycaemia

2.Hyperketonaemia

3.Metabolic acidosis

Clinical features [27]

(i) Polyuria, thirst, weight loss, weakness, nausea, vomiting, leg cramps, blurred vision and abdominal pain which is common in children

(ii) The striking features are those of salt and water depletion, with loss of skin tone, furred tongue, cracked lips, tachycardia, hypotension and reduced intra ocular pressure.

(iii) Cold extremities, tachycardia, air hunger (Kussmaul breathing), acetone odour in breath.

(iv) Hypothermia, confusion, drowsiness and coma.

2. Hypoglycaemia

It is the fall of blood glucose levels below 50-60mg/dl at which level symptoms occur in normal persons. Development of symptoms depends on the prevailing blood glucose levels and individual susceptibility.

Symptoms of hypoglycaemia

- ✓ Autonomic symptoms like palpitation, sweating, tremors, anxiety.
- ✓ Neuroglycopenic symptoms: tiredness, dizziness, drowsiness, difficult to concentrate and dysphasia.
- ✓ Lethargy, coma, convulsions
- ✓ Permanent brain damage.
- ✓ Sudden death due to cardiac arrhythmias (‘dead in bed’ syndrome).

3. Cardiovascular system

- ✓ Macroangiopathy
- ✓ Microangiopathy

4. Ocular complication

- ✓ Diabetic Cataract
- ✓ Diabetic Retinopathy
- ✓ Glaucoma develops in 6% cases.

5. Renal complications

- ✓ Renal arteriosclerosis.
- ✓ Pyelo nephritis - This is very common and may lead to chronic renal failure.
- ✓ Micro albuminuria
- ✓ Papillitis, Necroticans- The renal papillae will show necrosis, ultimately leading to viaemia.
- ✓ Kimmelstiel – Wilson syndrome (K.W. syndrome)
- ✓ This is develops in average 10 years duration clinically patient will present feature of nephritic syndrome.

6. Diabetic neuropathy

This is an early and common complication affecting 39% of diabetic patients. Metabolic neuropathy develops due to hyperglycaemia and this subsides with proper control of Diabetes.

- ✓ Peripheral neuritis (30%)
- ✓ Autonomic imbalance
- ✓ Diabetic amyotrophy
- ✓ Charcot's joint

7. Sexual and Genital Complications

Impotence and frigidity may develop erectile dysfunction in males is particularly important. Balanitis and Balanoposthitis are common complications in males. These are due to secondary infection as urine contains heavy amount of sugar and nitrogenous materials. Leucorrhoea may develop in females.

8. Pulmonary complications

Tuberculosis is very common in diabetes other infective complications like pneumonia, bronchopneumonia, pleurisy etc.

9. Effect on pregnancy and neonates

There may be miscarriages and abortions, toxaemias of pregnancy, hydramios etc Herculian child may be born of diabetic mothers due to secretion of excess of growth hormone.

10. Diabetic Foot

The Foot is a frequent site for complications in patients with diabetes, so foot care is particularly important. Tissue necrosis is common reason for hospital admission in diabetics may end in amputation.

TREATMENT

ANTI-DIABETIC DRUGS

INSULIN [28]

1. Insulin was first isolated from the Pancreas in 1922 by Banting and Best. Insulin is a polypeptide containing two chains of amino acids linked by disulfide bridges having molecular weight of 5808.

2. It is synthesized in the endoplasmic reticulum of the β cells as Preproinsulin that give rise to Proinsulin which undergoes peptic cleavage to form Insulin and C-peptide. C-peptide connects α and β chains.

3.The half-life of insulin in the circulation in humans is about 5 minutes and cleared from circulation within 10-15 minutes and stored in beta cell granules and discharged into interstitial fluid under appropriate stimulus, enters portal circulation and liver traps 50-60% and remaining enter peripheral circulation. It is degraded by enzyme Insulinase in liver, in the kidneys and muscles.

Preparations of insulin

Insulin preparation differs in their source and duration of action. Based on the source they may be classified as bovine, porcine and human insulins.

Figure 1:- Insulin

Table 3:- Insulin types

Conventional insulin	
▪ Short and rapid acting	Regular Semilente Lente
▪ Intermediate acting	Isophane insulin(NPH) Ultralente
▪ Long acting	Protamine zinc insulin
Highly purified insulins	
▪ Single peak insulin	Regular Lente
▪ Monocomponent insulins	Regular Lente
Human insulin	Regular Lente Ultralente Isophane

Insulin analogs ▪ Rapid acting ▪ Long acting	 Insulin lispro Insulin aspart Insulin glargine Insulin detemir
Insulin mixtures	Combinations of 20-50-% regular with 80-50% NPH (Neutral Protamine Hagedorn) insulins.

Insulin and its doses[29]

Regular/plane insulin	-	SOLUBLE INSULIN 40 IU/ml
LENTE INSULIN, ISOPHANE INSULIN, PROTAMINE ZINC INSULIN	-	40 IU/ml inj for sub-cutaneous use.
Highly purified regular	-	ACTRAPID MC-40,100 IU/ml inj
Highly purified lente Iu/ml	-	LENTARD, MONOTARD MC 40
Highly purified NPH	-	INSULATARD-40/ml
Mixture of regular + NPH insulin	-	MIXTARD 40IU/ml
Human actrapid	-	regular 40,100 IU/ml inj
Human monotard	-	Lente
Human insulatard	-	NPH
Human mixtard	-	Regular NPH
Humalog100U/ml inj 100 IU/ml (30ml vial)	-	NOVALOG 100 IU/ml inj, FLEXPAN

ORAL ANTIDIABETIC DRUGS [30]

The main disadvantage of insulin is the need for injection. The advance of oral hypoglycaemics came as a boon to millions of NIDDM patients with early and mild diabetes. Sulfonylureas were available in 1950s. We now have 5 groups of oral hypoglycaemics

- sulfonylureas,
- biguanides,
- meglitinides,
- alpha glucosidase inhibitors
- Thiazolidinediones.

Table 4:- Mechanism of action and adverse effects of oral antidiabetic drug

Mechanism of action and adverse effects of oral antidiabetics		
Oral antidiabetics	**Major mechanism**	**Adverse effects**
Sulfonylureas	↑ insulin release from pancreas ↑ tissue sensitivity to insulin	Hypoglycaemia Cholestatic jaundice Disulfiram like reaction
Biguanides	↓ hepatic gluconeogenesis ↑ tissue sensitivity insulin	Diarrhea, metallic taste, rarely lactic acidosis
Meglitinides	↑ insulin release from pancreas	Hypoglycaemia
Thiazolidinediones	↑ glucose transport into tissues ↓ hepatic gluconeogenesis	Weight gain, oedema, may precipitate CCF, risk of hepato toxicity
α glucosidase inhibitors	↓ glucose absorption ↓ hydrolysis of disaccharides	Flatulence, diarrhoea, abdominal distension

Status of anti-diabetic drugs

For the patients with IDDM, insulin is the only treatment as there is insulin deficiency due to destruction of β cells. Sulphonylureas need functional β cells for their action and therefore are not useful in IDDM. Uncomplicated NIDDM patients not controlled by diet and exercise are given oral hypoglycaemics. Mild NIDDM patients with recent onset diabetes, age above 40 years at onset of diabetes, obese with fasting sugar less than 200 mg/dl are candidates for oral hypoglycaemics.

Some of the other Siddha drugs used for diabetes mellitus

- Navalkottai Chooranam
- Seenthil Chooranam
- Sirukurinchan Chooranam
- Kompupagal Chooranam
- Thiripala Chooranam
- Navathanya Chooranam
- Santhiraganthi Chooranam
- Maruthampattai kashayam
- Atthiyathi kashayam
- Aanandhabairava Mathirai
- Mahalinga Mathirai
- Sanjeevi kirutham
- Meghaadi Kuligai
- Karpoorathi Mathirai
- Abragha parpam
- Naga parpam
- Velvanga parpam
- Kaanda sendooram

3.3. PHARMACEUTICAL REVIEW

Pharmaceutics is a discipline of pharmacy that deals with the process of turning a new chemical entity to be used safely and effectively by the patients.

Siddha pharmaceutics has very minute chemical processes in it. It has several chemical processes like purification of raw substances, grinding them with herbal juices for several days and subjecting the ground material to fire by way of *putam* process. Medicines prepared according to the above methods undergo several chemical changes.

Siddha medicines are classified into internal medicines (32) and external medicines (32). The drug taken for dissertation is in the form of *Mathirai.* Other names of *Mathirai* are *Kuligai, Urundai, and Vattam. Mathirai* comes under the category of internal medicines. [7b]

Purification of the drugs included

Purification of the drugs is mainly done to remove the toxicities, impurities like soil, dust, clay present in the drugs. Also the drugs when subjected to heat like roasting or soaked in liquids undergo certain chemical reactions such as oxidation of toxic substances to non-toxic, reduction of some poisonous chemicals to non-poisonous ones or undergo enzymatic reactions. In these ways, not only the toxicities and impurities are removed but also enhanced the potency of the drugs.

Concept and Terminology of pills

It is a pill prepared from a finely ground paste of drugs. The term *Mathirai* is the most fitting category of medicines as besides indicating the form of medicine that is pills. It also means that the minimal dosage unit is one pill (*Mathirai* means 1 unit). Preparation of *Mathirai* includes various processes like, extraction of juices, making decoction, preparing powders, grinding pastes and rolling into pills or pressing into tablets. The raw drugs are dried in the sun or shade and the drugs which are aromatic are to be roasted separately. The raw drugs are purified and grounded separately, then the compounded drug be grounded in a mortar for the prescribed period with the addition of prescribed juices and decoctions. If green drugs are to be added they should be made into fine paste before being used. Vegetable drugs which require

frying are fried and powdered. However, scented herbal drugs like cinnamon leaves and cloves, cinnamon bark are dried only in shade as otherwise their volatile oil are lost by drying.

The individual drugs should be separately weighed after being powdered and then taken in the prescribed ratio. After the pill mass has been prepared by following, the processes outlined in the recipe, it is convenient to roll it into long uniform pencils and then cut into bits of uniform length to give suitable pill weight and then rolling a pill from each piece. This is a fast process to prepare uniform pills as pinching and rolling every time is invariably a tiresome, tedious and time consuming messy process. The pill mass when rolled between the fingers should not stick. This is the correct consistency for rolling into pills. The pills should be always dried in a warm, dry shady place and never under the sun because volatile matters in the pills are easily lost and photochemical breakdown of active principles are faster in sunlight of the tropics. If the pill mass sticks to the fingers, a speck of ghee may be smeared on the fingers. Pills should be well dried in shade [31].

Storage and Usage

Almost all the *Mathirai* contain highly active ingredients. Hence they should be preserved in well stopper glass vials with relevant labels and instructions. If the *Mathirai* lose their natural shape, colours, smell, taste etc, it is not advisable to consume them. If properly stored, we can keep them for a year.

Preparation of *Mathirai* in Manufacturing Units

In the manufacturing unit, *Chooranam* is compressed into tablets. Tablets are unit forms of solid medicinal substances with or without suitable diluents prepared by compressing and they are mostly discoid in form. Binders like Gum acacia, lubricants like liquid paraffin and disintegrators like Talcum powder are used. *Chooranam* is first prepared according to the above procedures. Then the ingredients are mixed with in the form of granules before compressing as tablets. Too much fine powder refuses to form satisfactory tablets and so they must be mixed with some adhesive substances or binders such as gum acacia. To prevent the sticking of the tablets to the punches and dyes a lubricant like liquid paraffin is added. If the tablet is to dissolve quickly, a disintegrator like talcum powder is added [32].

Shelf life of the drugs

The shelf life of the drugs depends on the effectiveness of the preparation. The efficacy, smell, taste and appearance of the drugs gradually change as time goes on resulting in reduced potency thereby the desired effect is not attained. But some drugs appear to be good externally in spite of reduced efficacy. So they should not be considered for consumption and should be discarded. The shelf life of *Mathirai* is 1 year. According to recently published guidelines by Ayush, the shelf life period of *Mathirai* is 2 years. Also the following are the analytical parameters of specifications of *Mathirai* [33].

Table 5:- Testing parameters for *Mathirai*-AYUSH guidelines [34]

S.No	Tests
1	Description, Colour, Odour
2	Weight Variation
3	Disintegration Time (Not more than 15 minutes)
4	Identification TLC/ HPTLC/GLC
5	Assay
6	Test for heavy/toxic metals Mercury Arsenic Cadmium Lead
7	Microbial Contamination Total Bacterial count Total Fungal count
8	Test for specific pathogen E.coli Salmonella species Pseudomonas aeruginosa Streptococcus aureus
10	Test for aflatoxins B1, B2, G1, G2

Traditional tests for *Mathirai*

Characters :

- Non sticky on rolling.
- No cracks over the surface after drying.
- Shall be rolled uniformly over the plane surface.

Based on these characters the drug is assessed as the appropriate one for medication.

3.4. PHARMACOLOGICAL REVIEW

PHARMACOLOGICAL STUDY OF ANTI-DIABETIC ACTIVITY IN ANIMAL MODELS

Models for insulin dependent diabetes

1. Chemically induced diabetes

It is the most commonly used animal model of the diabetes mellitus. It is classified into three categories that include agents that

- ✓ Specifically damage the β-cell
- ✓ Cause temporary inhibition of insulin production
- ✓ Decrease the efficacy of insulin in target tissues
- ✓ In general chemicals in the first category are of interest as they reproduce lesions resembling IDDM [35].

2. Alloxan – induced diabetes

Alloxan is a cyclic urea analog and it was reported to produce permanent diabetes in animals.

Mechanism of action

The mechanism by which it induces diabetes is not very clear. Alloxan is highly reactive molecule that is readily reduced to diuleric acid, which is then auto oxidized back to alloxan resulting in the production of free radicals. These free radicals damage the DNA of the β cells and cause cell death. Second mechanism proposed for alloxan is its ability to react with protein SH group, especially the membrane proteins like glucokinase on the β cells; finally resulting in cell necrosis however, there are major species differences in response to alloxan [36].

Procedure

Animal models		**Dose**
Rabbits (2 to 3 kg)	–	150 mg /kg
Rats of wistar (150-200gm)	_	100-175mg/kg

Male beagle dogs (15-20kg)	–	60mg/kg
Monkeys	–	65-200mg/kg

All the animals which are administered with alloxan receive glucose and regular insulin for one week and food ad libitum. Then single daily dose of 28 IU insulin is administered. There is a triphasic change in the blood glucose level. At first there is a hyperglycemia at 2 hrs, then hypoglycemic phase at 8 hrs and finally an increase at 24 hrs.

3. Streptozotocin(STZ) - induced diabetes

STZ (2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose) is a broad spectrum antibiotic produced from the Streptomyces achromogens. The Diabetogenic activity of STZ was first described by Rakieten [37].

Mechanism of causing β cell damage

- ✓ By process of methylation
- ✓ Free radical generation
- ✓ Nitric oxide production

Procedure

STZ induces diabetes in all species of animals.

Animal model		**Dose**
Rats	-	50-60mg/kg
Mice	-	175-200mg/kg
Dogs	-	15mg/kg

After administration of the STZ the blood glucose level shows the triphasic change. Hyperglycemia at 1 hr, followed by hypoglycemia for 6hrs, and there is a stable hyperglycemia by 24-48 hrs.

4. Hormone induced diabetes

Dexamethasone, a long acting glucocorticoid is used to produce diabetes. Dexamethasone is administered to the rats in the dose of 2-5 mg/kg twice daily over a number of days.

5. Diabetes induced by the viral agents

Viruses are thought to be one of the etiologic agents for IDDM. Viruses may produce Diabetes mellitus by

- ✓ Infecting and destroying of β-cells in pancreas.
- ✓ A less infecting or cytologic variant producing a comparable damage by eliciting immune auto reactivity to the β-cells.
- ✓ Viruses producing systemic effect, not directly affecting β-cells.

Various numbers of viruses are used to induce diabetes. They are RN picorno virus, coxsackie-B4 (CB4), mengo – 2T, encephalomylocarditis (emc-d and m variants), double stranded RNA viruses, reovirus and lymphocytic choriomeningitis virus (LMCV). Primary isolates of these human pathogenic agents are generally not pancreatotrophic or ilytic to mouse b-cells and must be adapted for growth either by inoculation into suckling mice or by passage in cultured mouse β-cells [38].

6. Surgically induced diabetes

Surgical removal of all or parts of the pancreas induce diabetes; in partial pancreatectomy more than 90% of the organ is removed to produce diabetes. Depending on the amount of intact pancreatic cells, diabetes may range in duration from a few days to several months. Total removal of the pancreas results in an insulin dependent form of diabetes, and insulin therapy is required to maintain experimental animals. The portion of the pancreas usually left intact following a subtotal pancreatic resection is typically the anterior lobe or a portion thereof [39].

The use of pancreatectomy in combination with chemical agents, such as alloxan and STZ, produces a stable form of Diabetes mellitus in animals, such as cats and dogs, that does not occur when each procedure is applied an dependently. The combination therapy reduces the organ damage associated with chemical induction

and minimizes the interventions, such as enzyme supplementation, necessary to maintain a pancreatectomized animal.

7. Insulin antibodies-induced diabetes

Giving bovine insulin along with CFA to guinea pigs produces anti-insulin antibodies. Intravenous injection of 0.25-1.0 ml guinea pig anti-insulin serum to rats induces a dose dependent increase in blood glucose levels up to 300 mg%. This unique effect to guinea pig anti-insulin serum is due to neutralization of endogenous insulin by the insulin antibodies. It persists as long as the antibodies are capable of reacting with insulin remaining in the circulation. Slow i.v. infusion or i.p. injection prolongs the effect for more than a few hours. However, large doses and prolonged administration are accompanied by ketonemia, ketonuria, glycosuria and acidosis and are fatal to the animals. After lower doses, the diabetic syndrome is reversible after a few hours [40].

4. MATERIALS AND METHODS

4.1. PREPARATION OF THE DRUG

Selection of drug

The trial drug "*Linga Mathirai*" were prepared as per Siddha literature "*Skitcha rathina deepam.*"

Ingredients

*Lingam (*Cinnabar)	-	1 Palam (35gm)
Sivanar vembu (Indigofera aspalathoides)	-	5 Palam (175gm)
Naabi (Aconitum ferox)	-	1 Palam (35gm)
Nal Ennai (Gingelly oil)	-	5 Palam (175gm)

Collection of the drugs

The crude drugs such as Cinnabar, *Sivanar vembu*, *Naabi* were procured from Ramasamy chettiyar Raw Drug Stores at Parry's, Chennai.

Gingelly oil prepared by traditional method (Chekku) was purchased from nearby oil store in Aminjikarai, Chennai.

Identification and Authentication

All the raw drugs were identified and authenticated by Botanist and experts of Gunapadam Department (Pharmacology) at Government Siddha Medical College, Arumbakkam, Chennai.

The specimen samples of the identified raw drugs were preserved in the laboratory of P.G Gunapadam for future references.

Method of purification

Lingam

Lingam (35 gm) was soaked in Cow's milk (30 ml) in the mud plate for one day. The next day it was soaked in lemon juice (30 ml) in the same mud plate for one day. On third day it was washed thoroughly with pure water and dry it to get purified Cinnabar [41a].

Naabi

The root was treated with cow's urine (30 ml) for three days, the urine has been renewed every day [41b].

Method of preparation

Aconitum ferox (35 gm) had grounded well with water and made as paste, which was used to seal the 35 gm cinnabar. Then *Indigofera aspalathoides* of 175gm had also grounded with water made as paste and seal the above. The seal was covered by silk cloth kept it in sunlight for two hours. The gingelly oil (175 ml) was poured in iron pan, the above sealed one was fried until the gingelly oil has evaporates completely. Then the seals are removed, the cinnabar was grounded with boiled rice gruel of 1ml for three hours, then rolled it as pills. [43]

Preservation

The medicine was preserved in a clean, air tight container.

Administration of the drug

Form of the medicine	:	pills
Route of administration	:	Enteral
Dose	:	1 pills
Time of administration	:	2 times a day
Indication	:	**Diabetes** and Polyuria.

INGREDIENT:

Fig 2.1: ***Red sulphide of mercury***

Fig 2.2: ***Indigofera aspalathoides***

Fig 2.3: ***Aconitum ferox***

Fig 2.4 : Gingilly oil

Preparation of *Linga Mathirai*

Fig: 3.1. Sealed with silk cloth

Fig: 3.2. Fired with gingelly oil

Fig:3.3. Seal opened after processed

Fig:3.4. Grinding

Fig:3.5. Final form of *Mathirai*

Fig 4: Rolled pills of *Mathirai*

4.2. STANDARDISATION OF THE DRUG

World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care. The process of evaluating the quality and purity of herbo mineral drugs by means of various parameters like physical, chemical and biological observation is called standardization. Standardization of the this drug comes under the following categories

- Physio-chemical analysis
- Phyto chemical analysis
- Bio chemical analysis

ORGANOLEPTIC EVALUATION

The Organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odour, size etc.

1. Colour examination

Ten tablets were taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and results are noted.

2. Odour examination

Ten numbers of tablets were smelled individually. The time interval among two smelling was kept two minutes to overturn the effect of previous smelling. Odour of *Linga Mathirai* was noted in results table.

3. Size examination

The diameter of ten tablets was measured by Vernier caliper. The mean value of diameter was noted.

4.2.1. PHYSICO-CHEMICAL INVESTIGATION

Physico-chemical studies like total ash, water insoluble ash, acid Insoluble ash, loss on drying at 105°C and pH were done at, Central Research Institute, Chennai.

Solubility Test

A pinch of the sample (*Linga Mathirai*) was taken in a dry test tube and shaken well with distilled water. A little amount of the sample (*Linga Mathirai*) is shaken well with con. Hcl and then Con. H_2SO4. Solubility was observed.

Determination of Total Ash

About 2 g of the ground drug (*Linga Mathirai*) was accurately weighed in a silica dish and incinerated at a temperature not exceeding 450º until it was free from carbon, cooled and weighed. The percentage of ash with reference to the air-dried drug was calculated.

Determination of Water Soluble Ash

Total ash was heated up to 600^0C with 25 ml of distilled water for 10 minutes and the residue was ignited in the furnace to get a constant weight. And the weight was calculated.

Determination of Acid Insoluble Ash

The ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and insoluble matter was collected in an ash-less filter paper, washed with hot water and put up in flames to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was noted.

Determination of Moisture Content (Loss on Drying)

This procedure was done to determine the amount of volatile matter in the drug. A sample of 10 gram of the drug (*Linga Mathirai*) was placed in a tarred evaporating dish after accurately weighting without preliminary drying. The dish was dried at a temperature of 105^0C for about 5 hours and again weighed. The drying and weighing

procedure was repeated again and again until the difference between two successive weights was not more than 0.25%. And the weight was calculated.

pH value

Potentiometrically pH value was determined by a glass electrode and a pH meter. The pH of the *Linga Mathirai* was written in results column.

Tablet Disintegration test

Each *Linga Mathirai* was placed in each of the six tubes of the basket present in the disintegration apparatus. The apparatus was operated by using water as the immersion fluid maintained at 35-39 °C. At the end of the 30 min, the basket is lifted from the fluid and the state of the tablet is observed. The disintegration time of *Linga Mathirai* was recorded [44].

Weight variation test

It was carried out to make sure that, each number of tablets contains the proper amount of drug. The test was carried out by weighing the 20 tablets individually using analytical balance, then the average weight was calculated, and comparing the individual tablet weights to the average [45].

The percentage of weight variation is calculated by using this formula.

$$\% \text{ of wt. variation} = \frac{\text{Individual wt.} - \text{Average wt.}}{\text{Average wt.}} \times 100$$

Table 6:- Weight variation limits of Tablets (IP)

Average weight of tablets	Maximum percentage of weight difference allowed
80 mg or less	±10.0
Between 80 mg and 250 mg	±7.5
250 mg and more	±5.0

4.2.2. BIO-CHEMICAL ANALYSIS

The bio-chemical analysis was done to identify the acid and basic radicals present in the Linga Mathirai.

Preparation of extract

5g of *Linga Mathirai* was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

PRELIMINARY BASIC AND ACIDIC RADICAL STUDIES

TEST FOR BASIC RADICALS

1. Test for Potassium

To a pinch of the *Linga Mathirai* 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of *Linga Mathirai* extract, 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate**.**

3. Test for Magnesium

To 2ml of *Linga Mathirai* extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium

To 2ml of *Linga Mathirai* extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown colour.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the *Linga Mathirai*, made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. **Test for Iron (Ferrous)**

The *Linga Mathirai* extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour**.**

7. Test for Zinc

To 2 ml of the *Linga Mathirai* extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2m1 of the *Linga Mathirai* extract sodium hydroxide was added in drops and changes are noted.

9. Test for Lead

To 2 ml of *Linga Mathirai* extract 2ml of potassium iodide solution was added and noted for yellow coloured precipitate**.**

10. Test for Copper

a. A pinch of *Linga Mathirai* was made into a paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b. To 2 ml of *Linga Mathirai* extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11. Test for Mercury

To 2m1 of the *Linga Mathirai* extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

12. Test for Arsenic

To 2 ml of the *Linga Mathirai* extract 2ml of sodium hydroxide solution was added and observed for brown or red precipitate and noted.

TEST FOR ACID RADICALS

1. Test for Sulphate

To 2 ml of the *Linga Mathirai* extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The *Linga Mathirai* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. Test for Phosphate

The *Linga Mathirai* extract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. Test for Carbonate

The *Linga Mathirai* extract was treated with conc.Hcl and observed forth appearance of effervescence.

5. Test for Fluoride & Oxalate

To 2ml of *Linga Mathirai* extract 2ml of dil.acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

6. Test for Nitrate

To 1 gm of the *Linga Mathirai*, copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes.

4.2.3. MICROBIAL LOAD

AVAILABILITY OF BACTERIAL LOAD

Enumeration of bacteria by plate count – agar plating technique

The plate count technique is one of the most routinely used procedure because of the enumeration of viable cells by this method[46].

PRINCIPLE

This method is based on the principle that when material containing bacteria is cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies, therefore are the same as the number of organisms contained in the *Linga Mathirai*.

DILUTION

A small measured volume of *Linga Mathirai* are mixed with a large volume of sterile water called the diluent or dilution blank. Dilution are usually made in multiples of ten. A single dilution is calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluents}}$$

REQUIREMENTS

- Sample or Bacterial suspension
- 9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes(7)
- Nutrient agar medium (200 ml)
- Colony counter

PROCEDURE

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the *Linga Mathirai* extract into a 9 ml dilution blank labelled 10^{-1} thus diluting the sample 10 times.
3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
6. Repeat this procedure till the sample have been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are to used for each dilution.
8. Add approximately 15 ml of the nutrient medium, melted and cooled to 45^0c, to each petri dish containing the diluted Linga Mathirai extract. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidify.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c.

OBSERVATION

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies (average of 3 repleates)}}{\text{Amount of plated} \times \text{dilution}}$$

4.2.4. SOPHISICATED INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red)

Model	**:**	**Spectrum one: FT-IR Spectrometer**
Scan Range	**:**	**MIR 450-4000 cm-1**
Resolution	**:**	**1.0 cm-1**
Sample required	**:**	**50 mg, solid or liquid.**

It was the preferred method of infrared spectroscopy. FT-IR was an important and more advanced technique. It was used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It was an excellent tool for quantitative analysis [47].

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated.

In FT-IR infrared was passed from a source through a sample (*Linga Mathirai*). This infrared was absorbed by the sample(*Linga Mathirai*) according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the *Linga Mathirai*. Like the finger print there was no two unique molecular structures producing the same infrared spectrum. It was recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present [48].

SEM (SCANNING ELECTRON MICROSCOPE)

In scanning electron microscope high-energy electron beam was focused through a probe towards the sample (*Linga Mathirai*). Variety of signals was produced on interaction with the surface of the sample (*Linga Mathirai*). This results in the emission of electrons or photons and it was collected by an appropriate detector [49].

The types of signal produced by a scanning electron microscope include

- Secondary electrons
- back scattered electrons
- characteristic x-rays, light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample [50].

ICP-OES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Manufacturer: Perkin Elmer

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP)

Principle

An aqueous sample was converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which was a high temperature zone (8,000–10,000ºC). The analysts are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation was relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration was analysed by ICP-OES [51].

Application

The analysis of major and minor elements in solution *Linga Mathirai*.

Objectives

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements probes the outer electronic structure of atoms

Mechanism

In plasma emission spectroscopy (OES), a *Linga Mathirai* solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values [52].

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, and Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation

100 mg *Linga Mathirai* was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution. The digested *Linga Mathirai* solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

FTIR (Fourier Transform Infrared Spectroscopy):

Fig 5.1: FTIR INSTRUMENT

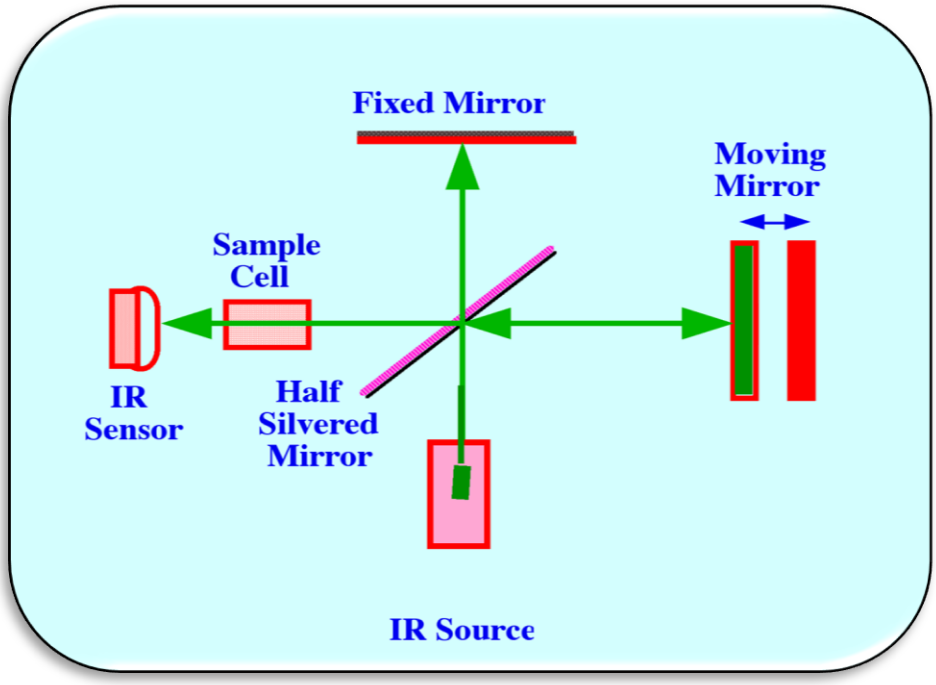


Fig 5.2: FTIR MECHANISM

SEM - SCANNING ELECTRON MICROSCOPE:

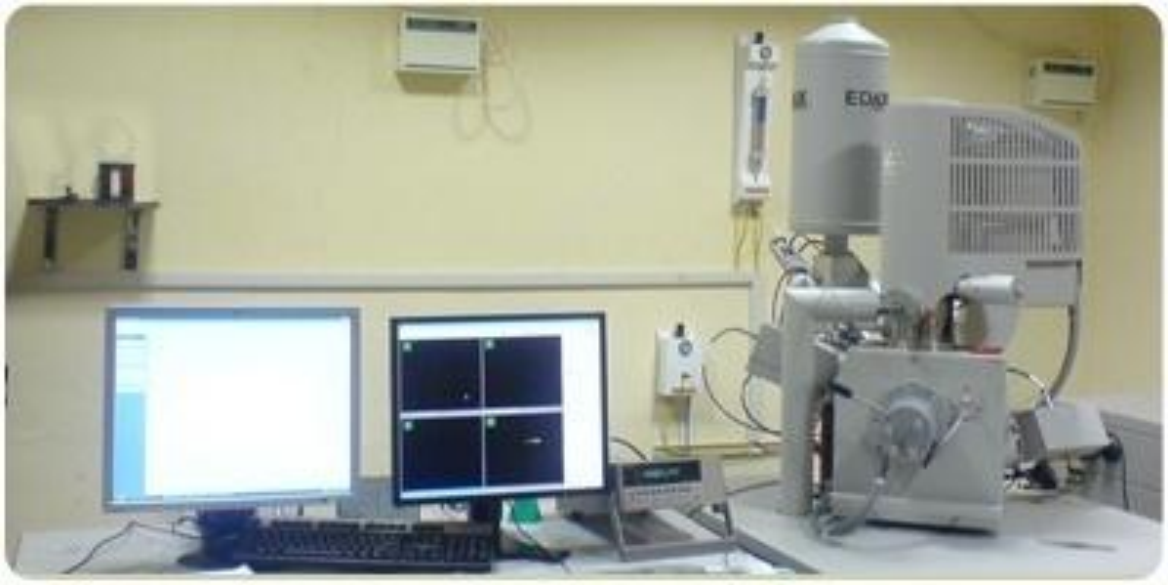


Fig 5.3: SEM INSTRUMENT

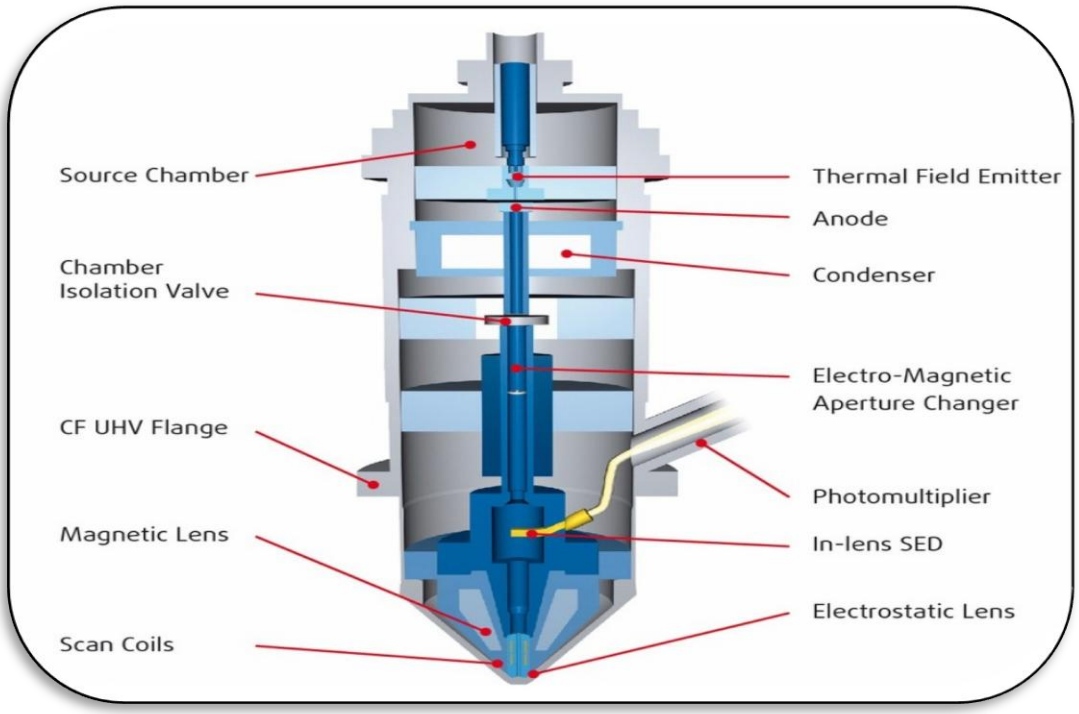


Fig 5.4: SEM MECHANISM

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY

Fig 5.5: ICP-OES ANALYSER (Perkin Elmer Optima 5300 DV)

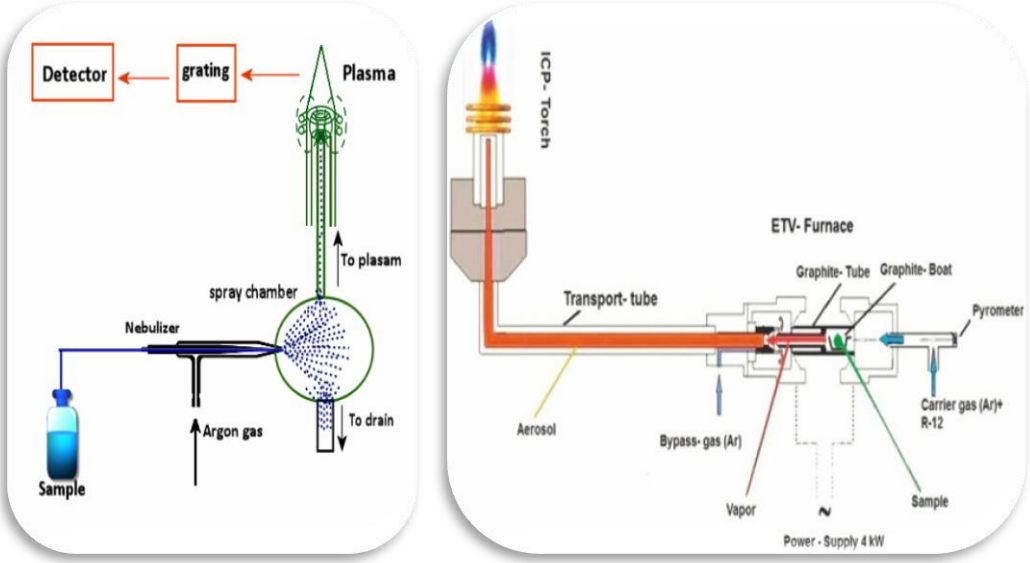


Fig 5.6: Mechanism of ICP-OES analyser

4.3. TOXICOLOGICAL STUDIES

ACUTE ORAL TOXICITY – OECD GUIDELINES – 423

INTRODUCTION

The acute toxic class method was a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co – operation and Development, Guideline-423.

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) under CPCSEA (Approval no: IAEC/XLIV/26/CLBMCP/2014) at C.L.Baid Metha College of pharmacy, Thuraipakkam, Chennai.

Animal: Healthy wistar albino female rat weighing 200–220 gm

PRINCIPLE

It was the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, information was obtained on the acute toxicity of the test substance to enable its classification. The substance was administered orally to a group of experimental animals at one of the defined doses. The substance was tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing was needed – dosing of three additional animals with the same dose – dosing of three additional animals at the next

higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes [53].

Studied carried out at three female rats under fasting condition, signs of toxicity was observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study.

METHODOLOGY

Selection of animal species

The preferred rodent species was rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strain Swiss albino rat were obtained from Animal house of king's institute, Guindy, Chennai. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within ±20 % of the mean weight of the animals. The studies were conducted in the animal house of C.L.Baid Metha College of pharmacy, Thuraipakkam, Chennai.

Housing and feeding conditions

The temperature in the experimental animal room should be 22ºC (+3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hrs dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

EXPERIMENT PROCEDURE

Administration of doses

Linga Mathirai was prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It was given in a single oral dose by gavages using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressiveness, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hours prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels

Since this *Linga Mathirai* has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step). Duration of study is 48 hours and evaluation of 14 days

Duration of Study: 48 hours

Evaluation: 14 Days

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material was likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body

weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

- The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hours.
- Special attention: First 1-4 hours after administration of drug.
- It was observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hours following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Body weights will be recorded at Day 1, 2, 7 and 14 of the study

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d. Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and necroscopic findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *Linga Mathirai* with 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique [54].

REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *LINGA MATHIRAI* ON RATS – (OECD-407 guidelines)

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that *Linga Mathirai* was non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route was considered to be a proposed therapeutic route [55].

Preparation and administration of dose

Linga Mathirai at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

OBSERVATIONS

Experimental animals were kept under observation throughout the course of study for the following

Body Weight

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality

All animals were observed twice daily for mortality during entire course of study.

Functional Observations

At the end of the 4th week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes. On 28th day of the experiment, 24 hours urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from fecal contamination. Toluene was used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations. On 29th day, the animals were fasted for approximately 18 hours, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities

of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Urine analysis

Urine samples were collected on end of treatment for estimation of normal parameters. The estimations were performed using appropriate methodology.

Necropsy

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Histopathology

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to Histopathological examination.

Statistical analysis

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet's multi comparision test using a computer software programme GRAPH PAD INSTAT-3 version.

4.4. PHARMACOLOGICAL ACTIVITY

4.4.1. ANTI-DIABETIC ACTIVITY OF *LINGA MATHIRAI*

Screening the drug *Linga Mathirai* against Streptozotocin (STZ) induced Diabetes in Wistar albino Rats

Experimental Animals

The animals were divided into 5 groups each constituting 6 rats. Group I were normal rats, Group II were STZ (55 mg/kg b.w., i.p) induced diabetic rats. Group III STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with Glibenclamide 5mg/kg b.w/p.o Group IV STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with *Linga Mathirai*200mg/kg b.w/ p.o Group V STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with *Linga Mathirai* 400mg/kg b w/p.o for 28 days.

Methodology

Induction of Diabetes

Diabetes was induced in male Wistar albino rats aged 2–3 months (180–200 g body weight) by intra peritoneal administration of STZ (single dose of 55 mg/kg b.w) dissolved in freshly prepared 0.01 M citrate buffer, pH 4.5.

After injection the animals had food and water *ad libitum* and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. The development of diabetes was confirmed after 72 hours of the Streptozotocin injection. After 72 hours of STZ injection under mild anesthesia the blood was withdrawn from the tip of the tail of each rat and the blood glucose level was analyzed. Animals with more than 250 mg/dl was considered as diabetic.

Fasting blood glucose levels was measured before the administration of extracts. The blood glucose levels were checked on 0th, 7th, 14th, and 21st day of the treatment period. Blood was collected from snipping of the rat tail. Blood glucose levels were measured [56].

Experimental Design

Diabetic rats were divided into three groups with six animals in each group.

Table 7:- Experimental design

Groups	Treatment
Group I	Normal Control
Group II	Diabetic control- STZ (55 mg/kg)
Group III	Diabetic control- Glibenclamide (5 mg/kg)
Group IV	Diabetic control- *Linga Mathirai* 200mg/kg
Group V	Diabetic control- *Linga Mathirai*400mg/kg

Blood collection

All the experimental rats were fasted overnight and the blood was withdrawn through puncturing retro orbital sinus on the 5^{th} day, 15^{th} day and 20^{th} day of post induction period to determine blood glucose level by GOD-POD kit method. The change in body weight was observed throughout the treatment period in experimental animals [57]

Statistical Analysis

All the values were expressed as Mean ± S.D. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet's t test. $P<0.05$ were considered significant.

4.2.2. ANTI – DYSLIPIDEMIC ACTIVITY

TRITON WR 1339 INDUCED DYSLIPIDEMIA FOR *LINGA MATHIRAI*

Experimental protocol

Total number of groups:	4
Number of animals / group:	6
Sex:	Both
Strain:	Wistar albino rats
Body Weight:	200 – 250 g

Surfactant administration

Surfactant:	10% Triton WR – 1339
Route of administration :	Intravenously
Vehicle:	Saline

Test drug administration

Vehicle:	2% CMC
Route of administration :	Oral
Drug dose:	*Linga Mathirai* - 200 mg/kg b.wt

Procedure

Wistar rats weighing 200–350 g were starved for 18 hours and then injected intravenously with 10% Triton WR 1339 (isooctyl-polyoxyethylene phenol).

Phase I: Serum cholesterol levels increase sharply 2–3 times after 24 hours.

Phase II: The hypercholesterolemia decreases nearly to control levels within the next 24 hours.

The test drug (*Linga Mathirai)* employed or the solvent for the controls are administered simultaneously with the Triton injection or 22 hours thereafter. Serum cholesterol analyses are made 6, 24, and 48 hours after Triton injection.

Mechanism

The mechanism of the Triton induced hypercholesterolemia in phase I was thought to be due to increased hepatic synthesis of cholesterol through the ability of Triton to interfere with the uptake of plasma lipids by the tissues. Drugs interfering with cholesterol biosynthesis were shown to be active in phase I, while drugs interfering with cholesterol excretion and metabolism were active in phase II [58].

Experimental design

The animals were divided into three groups with six animals in each group.

Group I → Normal control administered with 2% CMC

Group II → Dyslipidemic control received 10% Triton WR – 1339

Group III → Standard administered with Lovastatin (10 mg/kg b.wt) and triton

Group IV → Test drug *Linga Mathirai* - 200mg/kg b.wt

All the animals after 72 hours of triton injection (ie. after inducing dyslipidemia) the respective treatment was continued for 7 days.

Collection of blood

On the 8th day the blood was collected by retro orbital sinus puncture, under mild ether anesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and it is used for various biochemical experiments. Then animals were sacrificed and collected the liver.

Biochemical analysis

- Total cholesterol
- High-density lipoprotein
- Low-density lipoprotein
- Very low density lipoprotein
- Triglycerides

4.2.3. ANTI-OXIDANT ACTIVITY OF *LINGA MATHIRAI* (In-Vitro study)

FREE RADICAL SCAVENGING ACTIVITY

DPPH Assay (2, 2-diphenyl -1-picrylhydrazyl)

The radical scavenging activity of extracts was determined by using DPPH assay according to Chang et al [2001].The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used.

Principle

1, 1-diphenyl-2-picryl hydrazyl was a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability [59].

Reagent preparation

0.1mm DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Procedure

Different volumes (2.5µl - 40µl) of plant extracts were made up to a final volume of 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control.

Calculation

$$\% \text{ inhibition} = \frac{\text{Control} - \text{test}}{\text{Control}} \times 100$$

5. RESULTS AND DISCUSSION

Many studies have been carried out to bring the efficacy and potency of the drug ***Linga Mathirai***. The study includes literary collections, organoleptic character, physicochemical, instrumental analysis, toxicological study and pharmacological study. The drug ***Linga Mathirai*** has been selected for Anti-Diabetic activity in reference with the text ***"Skitcha Rathina Deepam".***

- Literary collections about the drug from various text books were done. Siddha literatures related to the drug bring the evidence and importance of its utility in treating the diabetes.
- Gunapadam review brings the effectiveness of the ingredients present in the drug for Anti-diabetic, Anti-dyslipidemic and Anti-oxidant properties.
- Botanical aspect explains the identification, morphological description, microscopic character, macroscopic character, active principle and medicinal uses of the plants.
- Siddha and modern aspect of disease explains about clinical features and complication of Diabetes mellitus and also explains adverse effect of drugs used in modern aspect.
- Pharmaceutical review describes about the purification, preparation, administration of *Mathirai* and explains about its self life properties.
- The pharmacological review explains about the methodology of Anti-Diabetic Activity and the drugs used.

STANDARDIZATION OF THE TEST DRUG

Standardisation of the drug is more essential to derive the efficacy, potency of the drug by analysing it by various studies. Following are the results of physicochemical. Physical characterisation and estimation of basic and acidic radicals have been done and tabulated.

Toxicological results of the drug and pharmacological activity of the drug were derived. Its result has been tabulated and interpretation was made below. Thus it is to give a complete justification to bring the effectiveness of the trial drug *Linga Mathirai*.

ORGANOLEPTIC CHARACTER

Table 8 :- Physical characterization of *Linga Mathirai*

S.NO	Parameter	Results
1	Colour	Brick red
2	Odour	Odourless
3	Consistency	Hard
4	Shape	Spherical
5	State of matter	Solid
6	pH	7.6
6	Total ash	1.379%
7	Water soluble Ash	0.294%
8	Acid insoluble Ash	0.871%
9	Loss on Drying at 105^0C	0.483%
10	Disintegration time	23 min

Discussion

- pH of *Linga Mathirai* is 7.6. It is slightly alkaline in nature. The alkaline medium enhance the mineral storage in order to buffer, reduces aging process, increase the utilization of oxygen level in body [60].
- The amount of minerals and earthy materials present in the drug are represented by Total ash value. The value of *Linga Mathirai* is 1.379%, it determines the purity of the drug.
- Water soluble ash represents easy facilitation of diffusion and osmosis mechanism. Here the value of Linga Mathirai is 0.294% will denotes its diffusion capacity.
- The amount of siliceous matters in the drug are represented by acid insoluble ash value. The acid insoluble ash value of *Linga Mathirai* is 0.871%, which determines the superior quality of the *Linga Mathirai*.

- The moisture content of the drug is determined by loss on drying. These will also indicates stability and shelf life of the drug. Here the percentage denotes the higher stability of the *Linga Mathirai*.
- *Linga Mathirai* is formulated according to classical Siddha text, disintegration indicates the better solubility and absorbability of drug.

Weight variation test

Table 9:- Uniformity weight variation test result of *Linga Mathirai*

S.No.	Weight of each Mathirai (mg)	% of weight variation	Maximum weight variation with in ±7.5%	Maximum weight variation with in ±15.0%
1	138	5.423%	Yes	Yes
2	137	4.660%	Yes	Yes
3	132	0.840%	Yes	Yes
4	130	-0.687%	Yes	Yes
5	139	6.187%	Yes	Yes
6	135	3.132%	Yes	Yes
7	126	-3.743%	Yes	Yes
8	129	-1.451%	Yes	Yes
9	125	-4.507%	Yes	Yes
10	133	1.604%	Yes	Yes
11	127	-2.979%	Yes	Yes
12	128	-2.215%	Yes	Yes
13	136	3.896%	Yes	Yes
14	124	-5.271%	Yes	Yes
15	129	-1.451%	Yes	Yes
16	131	0.076%	Yes	Yes
17	135	3.132%	Yes	Yes
18	120	-8.326%	No	Yes
19	122	-6.799%	Yes	Yes
20	126	-3.743%	Yes	Yes
	Average : 130.95 g			

Discussion

- Average weight of the *Mathirai* was noted as 130.95g. Out of 20 tablets tested, 19 tab of them lies within ±7.5% weight variation (1 tab above the limit) and all 20 tab lies within ±15% weight variation.
- According to the limits of weight test cited in the Indian pharmacopoeia, ***Linga Mathirai*** passed the Uniformity weight test.
- The uniformity test resembles uniformal distribution of this tablet helps good absorbtion and distribution.

TRADITIONAL TEST FOR PILL

Character	Inference
Non sticky on rolling	+
No cracks over the surface after drying	+
Shall be rolled uniformly over the plane surface	+

BIO CHEMICAL ANALYSIS

Basic radicals

Table 10:- Results for Basic radicals

S.NO	Parameter	Observation	Result
1	Test for Potassium	Yellow colour precipitate	+ve
2	Test for Calcium	-	-ve
3	Test For Magnesium	-	-ve
4	Test For Ammonium	-	- ve
5	Test For Sodium	-	-ve
6	Test for Iron (Ferrous)	-	-ve
7	Test For Zinc	-	-ve
8	Test For Aluminium	-	-ve

9	Test For Lead	-	- ve
10	Test for Copper	-	- ve
11	Test For Mercury	-	- ve
12	Test for Arsenic	-	- ve

Interpretation

The basic radical test shows the presence of Potassium**,** and absence of heavy metals.

Potassium

The K+ inwardly rectifier channel (KIR) is one of the two sub-units of the pancreatic islet ATP-sensitive potassium channel complex (IKATP). It has a key role in glucose-stimulated insulin secretion and thus is a potential candidate for a genetic defect in Type II (non-insulin-dependent) diabetes mellitus [61].

Acid radical

Table 11:- Results for Acid radicals

S.NO	Parameter	Observation	Result
1	Test for Sulphate	Formation of white precipitate	+ ve
2	Test for Chloride	-	- ve
3	Test for Phosphate	-	- ve
4	Test for Carbonate	-	- ve
5	Test for fluoride &oxalate	-	- ve
6	Test For Nitrate	-	- ve

Interpretation

The acidic radicals test shows the presence of sulphate.

MICROBIAL LOAD

Availability of bacterial and fungal load in *Linga Mathirai*

Bacteria

Fig 5.1. 10^{-4} dilution

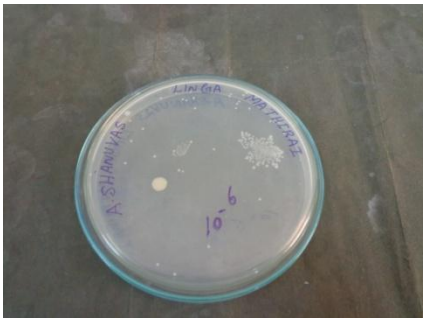


Fig 5.2. 10^{-6} dilution

Fungi

Fig 5.3. 10^{-2} dilution

Fig 5.4. 10^{-3} dilution

- The contaminated toxins present in the drug will produce adverse effect, which develops unwanted diseases. They are unfit for humans [62].
- Here, the contamination of *Linga Mathirai* have been examine by bacterial and fungal load.
  - ✓ Total bacterial load in 10^{-4} dilution is 4 and in 10^{-6} dilution is Nil.
  - ✓ Total fungal load in 10^{-2} dilution is Nil and in 10^{-3} dilution is Nil.
- The load of bacterial and fungal are within the limits of WHO norms.

INSTRUMENTAL ANALYSIS

FTIR

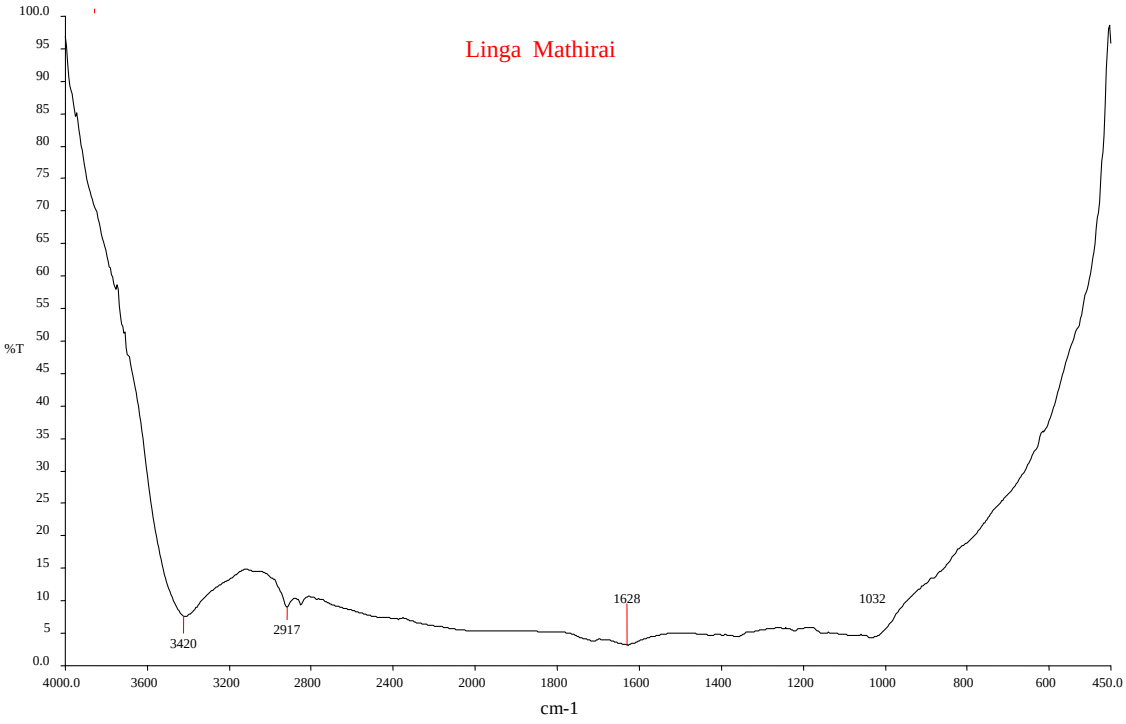


Table 12:- FTIR Result of *Linga Mathirai*

CHARACTERISTIC ABSORPTION(S) (cm^{-1})	FUNTIONAL GROUPS
3420	Alcoholic group Normal ‘‘polymeric’’ OH stretch Amine group of N-H Bending
2917	Alkane C-H Stretch, Acid group of O-H stretch
1628	Alkene group of C=C stretch, Amide group of N-H Bending
1032	Alkyl Halide C-F stretch, Ether C-O stretch, Ester C-O stretch

DISCUSSION

- The above table shows the presence of Alcoholic group, Amine group, Alkane group and Alkyl Halide are the organic functional groups, also Ether and Ester group of Carbonyl functional groups.
- OH group has higher potential towards inhibitory activity against microorganisms.

SCANNING ELECTRON MICROSCOPY (SEM)

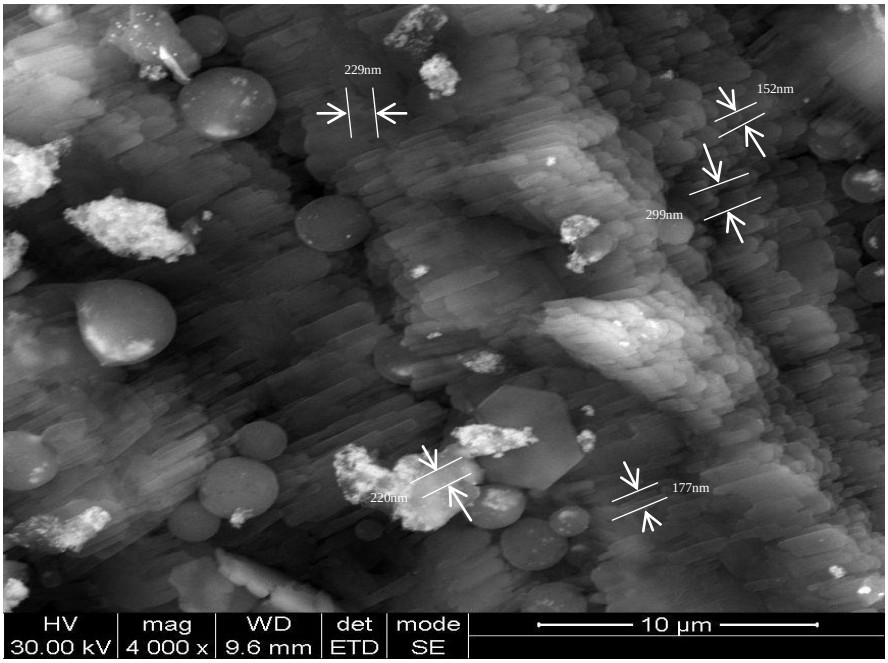


Fig 6.1. SEM image of 10 µm

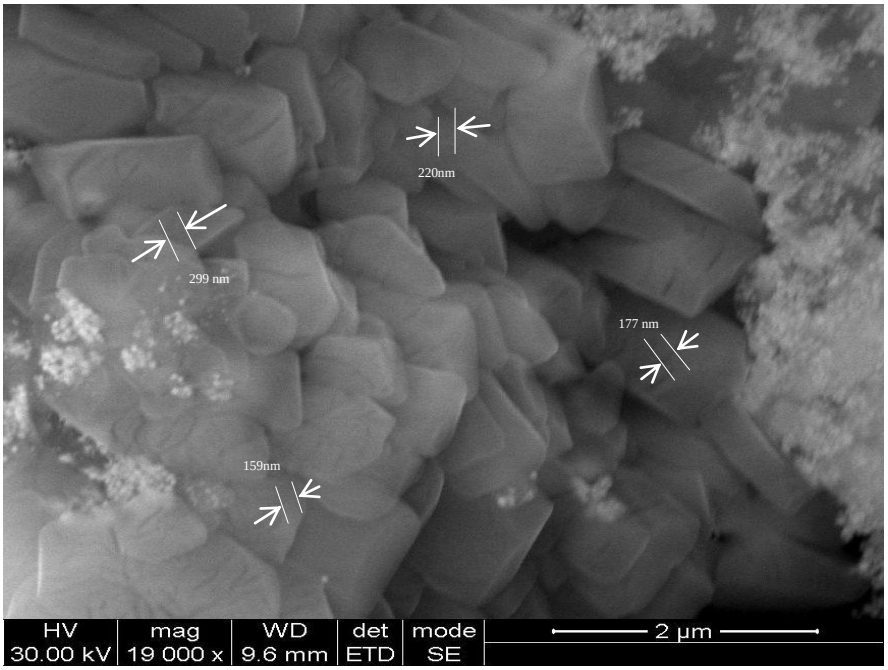


Fig 6.2. SEM image of 2 µm

Scanned electron microscope image of *Linga Mathirai*

DISCUSSION

SEM picture shows Nano and micro particle (Ultra fine particle) size of the sample. And the picture shows various sizes of the particles like 152nm, 177nm, 220nm, 229 and 299nm.

Sizes ranging from 179nm to 304nm. The surface of the sample grains is uniformly arranged. These are micro particles presenting as 152nm, 177nm, 220nm, 229nm and 299nm. The difference in morphology as evident from the micrograph is due to presence of chemicals in the samples.

Micro particles-significance

- Micro particles are defined as particulate dispersion or solid particles with a size in the range of 100-1000nm in diameter.
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting.
- They control the release of drug during the transportation and at the site of localization, alters drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby bio-availability and reduced side effects [63].

ICP-OES

Table 13:- ICP-OES Result of *Linga Mathirai*

S. no	Elements	Detected levels
1.	Sulphur	52.514 mg/L
2.	Potassium	20.821 mg/L
3.	Calcium	12.120 mg/L
4.	Phosphorus	08.541 mg/L
5.	Mercury	0.674 mg/L
6.	Iron	0.380 mg/L
7.	Sodium	03.110 mg/L
8.	Arsenic	BDL
9.	Cadmium	BDL
10.	Nickel	BDL
11.	Lead	BDL

DISCUSSION

From the above results, the heavy metals like Arsenic, Cadmium and Lead are below detectable limit.

Mercury was in permissible limit.

Hence, the safety of the drug *Linga Mathirai* is ensured.

Also, the drug contains Calcium, Iron, Potassium, Sodium, Sulphur and Phosphorus.

Calcium [64]

- Calcium was associated with the lower risk of Diabetes mellitus
- Calcium is necessary in normalising the glucose tolerance.
- Abnormal regulation of intracellular calcium affecting both insulin sensitivity and insulin release.

Iron

- The heme containing enzymes such as catalase and peroxidase protect cell against potentially damaging highly reactive species.
- Iron is essential for many numbers of biological functions such as growth, reproduction, healing and immune function.

Sodium

- Recently sodium glucose cotransporter 2 reabsorbs most of the glucose filtered by the kidneys. Thereby, lowering the blood glucose levels and have been approved as new anti hyperglycemic drug. [65].

A synergistic effect of all these Calcium, Iron, Potassium, Sodium, Phosphorus and Sulphur increases the potency of the drug against Diabetes mellitus.

TOXICOLOGY STUDIES

ACUTE ORAL TOXICITY IN RATS– OECD 423

Wistar albino rat was treated with the test drug *Linga Mathirai* of single dose of 2000mg/kg in 2%CMC as suspention. This study was conducted as per the OECD guidelines. The result of acute toxicity of *Linga Mathirai* has been tabulated below.

Table 14:- Observation in acute toxicity study

Group	Day
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant colour change
Piloerection	Normal
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Urination	Normal

Table 15:- Dose finding experiment and its behavioural Signs of Toxicity for *Linga Mathirai*

Dose mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
2000	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1.*Alertness 2.Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9 Convulsions 10. Muscle Spasm 11. Catatonia 12.Musclerelaxant 13.Hypnosis 14.Analgesia15.Lacrimation 16. Exophthalmos 17.Diarrhoea 18. Writhing 19 Respiration 20. Mortality*

Discussion

In the acute toxicity study, the rats were treated with different concentration of *Linga Mathirai* from the range of 5mg/kg to 2000mg/kg which did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period.

These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract. In acute toxicity test the *Linga Mathirai* was found to be non toxic at the dose level of 2000mg/ kg body weight.

RESULTS OF SUB-ACUTE ORAL TOXICITY 28-DAYS REPEATED DOSE STUDY IN RATS

Wistar albino rat was treated with the test drug *Linga Mathirai* for 28 days repeated dose of 100mg/kg and 200 mg/kg in 2% CMC as suspention. This study was conducted as per the OECD guidelines. The result of sub acute toxicity of *Linga Mathirai* has been tabulated below

Body Weight

Table 16:- Effect of *Linga Mathirai* on Body Weight of rats

Dose (mg/kg/day)	Days				
	0	**7**	**14**	**21**	**28**
Control	120.59±0.92	122.79±0.87	123.52±1.18	127.24±1.12	131.25±1.05
100	126.02±1.15	129.86±1.57	132.94±1.51	135.77±0.76	136.78±1.14
200	127.49±1.24	130.92±0.89	133.83±1.53	135.98±0.92	138.81±0.81

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

Organ weight

Table 17:- Effect of *Linga Mathirai* on Organ weight of rats

Organ	Control	100 mg/kg	200 mg/kg
Liver (g)	5.24±0.14	5.1±0.24	5.2±0.3
Heart (g)	0.70±0.05	0.65±0.12	0.72±0.11
Lung (g)	1.78±0.25	1.66±0.42	1.73±0.4
Spleen (g)	0.74±0.07	0.68±0.17	0.78±0.08
Brain (g)	1.43±0.18	1.2±0.41	1.45±0.36
Kidney (g)	0.70±0.05	0.61±0.11	0.69±0.09

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

Haematological parameters

Table 18:- Effect of *Linga Mathirai* on Haematological parameter of rats

Parameter	Control	100mg/kg	200 mg/kg
RBC(x 10^6/mm^3)	8.29±0.43	9.25±0.46	9.61±0.40
PCV (%)	49.66±0.77	50.92±0.73	52.22±1.06
Hb (%)	15.13±0.39	15.69±0.37	15.86±0.24
WBC(x 10^3/mm^3)	11.75±0.85	10.35±0.34	9.46±0.34
Neutrophils (%)	23.29±0.73	26.91±1.24	28.13±1.14
Eosinophills (%)	4.1±0.23	3.43±0.52	2.70±0.64
Lymphocyte (%)	85.5±0.46	85.78±0.21	86.5±0.41
Platelets(x 10^3/mm^3)	425.73±1.35	427.02±0.98	424.54±2.19

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

Biochemical parameters

Table 29:- Effect of *Linga Mathirai* on Biochemical parameter in rats

Parameters	Control	100 mg/kg	200 mg/kg
Glucose (mg/dl)	108.63±0.81	107.14±0.56	105.95±0.78
BUN (mg/dl)	22.06±1.55	24.45±1.86	25.70±2.39
Creatinine (mg/dl)	0.85±0.07	0.83±0.06	0.76±0.03
SGOT (U/L)	74.35±1.23	72.93±1.15	71.43±1.24
SGPT(U/L)	27.07±0.84	25.49±1.17	23.94±0.97
ALP (U/L)	104.63±1.14	103.43±1.69	102.71±0.71
Protein (g/dl)	8.58±0.68	8.24±0.53	7.66±0.43
Albumin (g/dl)	5.34±0.40	4.77±0.31	4.11±0.14
Total Cholesterol (mg/dl)	93.21±1.16	92.01±0.64	90.02±1.02
Triglycerides (mg/dl)	52.58±1.56	55.99±1.46	64.23±1.59

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

Urine Parameter

Table 20:- Effect of *Linga Mathirai* on Urine parameter in rats

Parameter	Control	100 mg/kg	200 mg/kg
Colour	Yellow	Yellow	Yellow
Transparency	Clear	Clear	Turbid
Specific gravity	1.01	1.02	1.04
pH	7.2	7.4	6.9
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	-ve	-ve
Blood	Absent	Absent	Absent
RBCs	Nil	Nil	Nil
Epithelialcells	Nil	Nil	Nil
Casts	Nil	Nil	Nil

DISCUSSON

Body weight

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited overall mild weight gain throughout the dosing period of 28 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

Organ weight

The weights of organs recorded did not show any significant differences in the treatment and the control group indicating that *Linga Mathirai* was not toxic to kidney, liver and spleen

Haematological investigation

There was no significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Biochemical investigation

No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.

Urine analysis

Urine analysis data of control group and the test groups of animals taken on 28^{th} day showed no abnormal results.

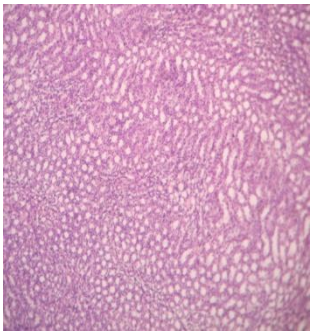
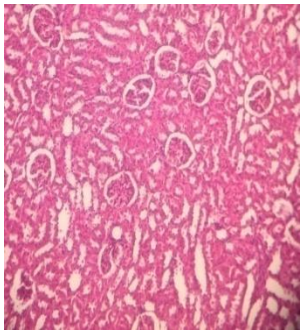
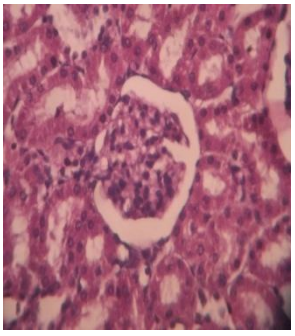
The dose selected for the sub acute toxicity study was 100mg, 200mg/kg of *Linga Mathirai*. All the animals were free of intoxicating signs throughout the dosing period of 28 days.

- ✓ No physical changes were observed throughout the dosing period.
- ✓ No mortality was observed during the whole experiment.
- ✓ No abnormal deviations were observed.

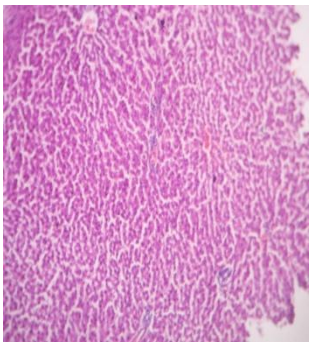
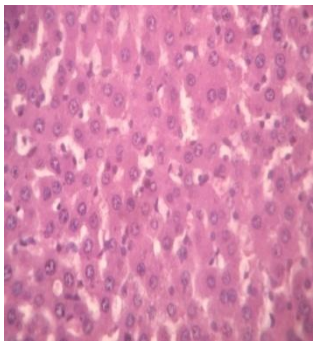
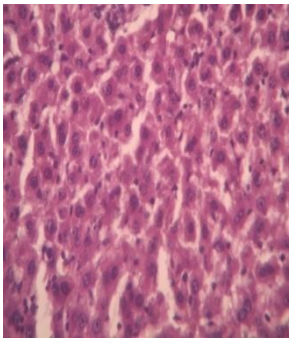
Fig 7. HISTOPATHOLOGY SLIDES:

Control	*Linga Mathirai* 100m	*Linga Mathirai* 200mg

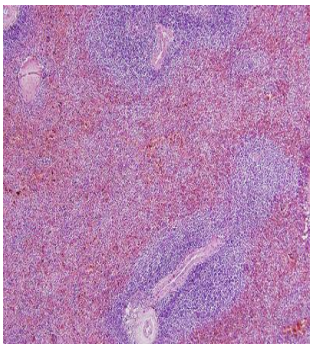
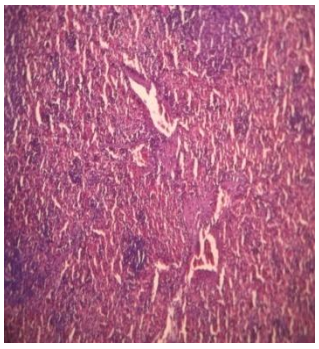
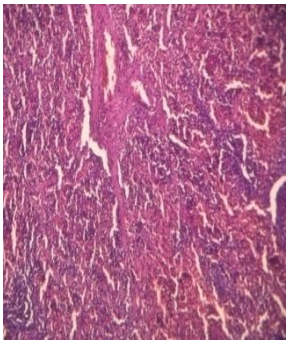
KIDNEY

LIVER

SPLEEN

DISCUSSION

From histopathological examinations, the slides of animal's organ did not reveal abnormalities.

From the acute and sub-acute toxicity studies the drug produced some significant changes .But the values were found within normal limits. So the drug *Linga Mathirai* was nontoxic and safe.

Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

PHARMACOLOGY STUDIES

ANTI DIABETIC ACTIVITY

Blood glucose level

Table 21:-Effect of *Linga Mathirai* on Blood glucose level in Streptozotocin induced diabetic rats

Group	Blood glucose (mg/dl)				
	Day – 0	**Day – 7**	**Day – 14**	**Day – 21**	**Day – 28**
Normal control	89.40±1.13	87.53±1.32	92.75±2.87	85.13±2.43	94.70±2.11
Diabetic Control	106.22±1.14	240.9±1.66	275.3±1.41	287.8±1.32	295.4±1.20
Diabetic rats + Glibenclamide	103.5±1.45	151.0±1.32**	142.05±1.33	114.3±1.48	102.3±1.45**
Diabetic rats +Linga Mathirai 100mg/kg	99.13±1.22*	220.5±1.00	190.2±2.17	161.8±1.20	129.5±1.44
Diabetic rats +Linga Mathirai 200mg/kg	94.23±1.38*	179.8±1.34	158.04±1.92	131.03±1.76	114.8±1.87*

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

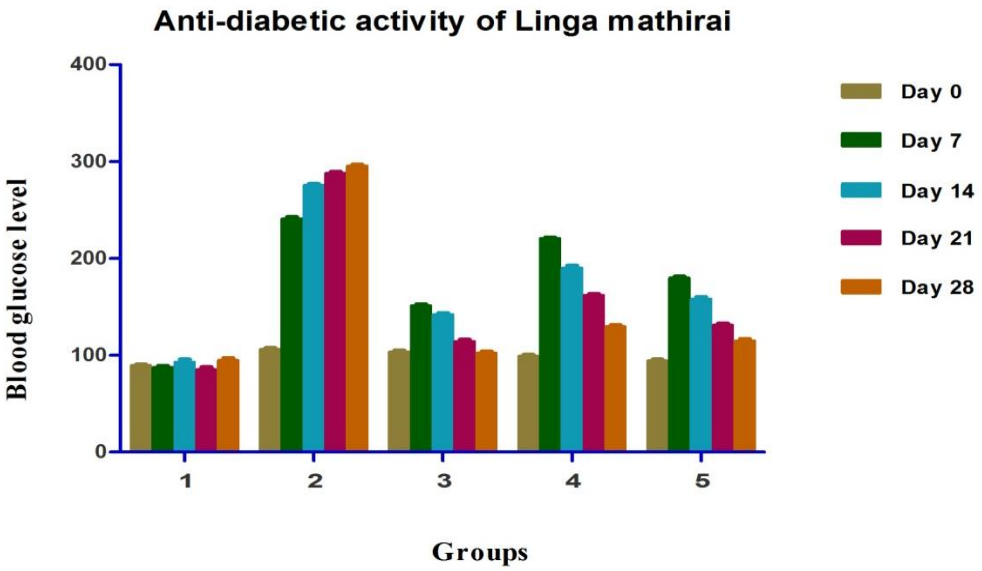


Chart 1:- Anti Diabetic activity of *Linga Mathirai*

Body weight in Streptozotocin induced diabetic rats

Table 22:- Effect of *Linga Mathirai* on Body weight in Streptozotocin induced diabetic rats

Group No.	Treatment	Body weight (g) on post induction days			
		Initial	5th day	15th day	20th day
I	Normal control	163.65±2.52	165.39±3.21	166.97±2.58	168.95±2.45
II	Diabetic Control	159.59±2.78	157.70± 3.34	129.30±2.10	120.20±2.33
III	Diabetic rats + Glibenclamide	161.30±3.26	163.40 ± 2.77*	167.8±3.26	174.70 ± 2.37**
IV	Diabetic rats + Linga Mathirai 200mg/kg	160.90±4.78	164.20 ± 3.23	171.20 ± 4.86*	176.20± 4.89**

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

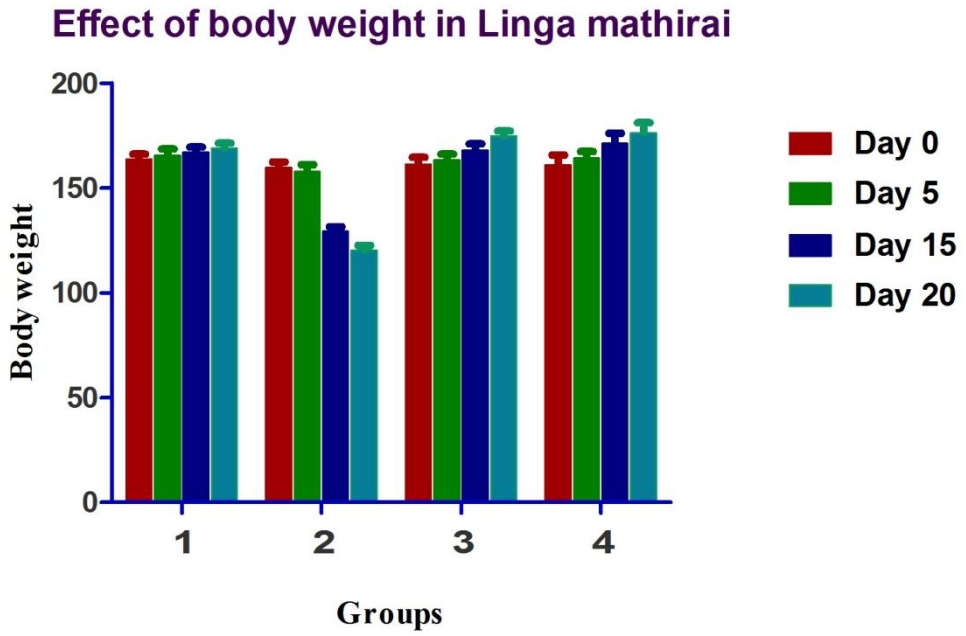


Chart 2:- Body weight in Diabetic induced rats

DISCUSSION

Anti Diabetic activity

The Anti-Diabetic Activity of the test drug *Linga Mathirai* has been estimated in the streptozotocin induced diabetes in Wistar albino rat.

Administration of the streptozotocin effectively induced diabetes mellitus in the animal model which is known by the increased glucose level.

STZ, slightly cytotoxic agent of pancreatic beta cells, selectively destroys the pancreatic insulin secreting beta cells, thus leaving less active cells and resulting in diabetes mellitus. Thus it is widely used to induce diabetes in animal models. It also interferes with cellular metabolic oxidative mechanisms [66].

Oral administration of the test drug *Linga Mathirai* taken in the dose of 200mg/kg showed significant decrease in the sugar level.

The possible mechanism by which the test drug *Linga Mathirai* brings about a decrease in the blood sugar may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β-cells of the islets of Langerhans or its release.

Linga Mathirai a Siddha herbal formulation used in the treatment of diabetes shows significant reduction in the sugar level which was studied by inducing diabetes in the animal model Wistar albino rat by streptozotocin.

Body weight changes

Body weight of the animal models in this study was also monitored. There is decrease in the body weight of the animal treated with the control. Whereas the animal treated with *Linga Mathirai* shows a significant improvement in the body weight. Thus the trial drug not only reduced the sugar level but also maintains the body weight in Diabetes Mellitus.

ANTI DYSPIPIDEMIC ACTIVITY

Table 23:- Effect of *Linga Mathirai on* lipid profile of Triton induced Dyspipidemia in rats

Lipid parameters	Group-I Normal control	Group-II Triton control rats	Group-III Dyspipidemic+ Lovastatin	Group-IV Dyspipidemic+ *Linga Mathirai*
TotalCholesterol (mg/dl)	71.49±0.91	172.46±1.14	66.08±1.32	73.50±1.03
HDL (mg/dl)	30.64±1.11	26.20±1.07	62.92±1.58**	73.4±1.29*
LDL (mg/dl)	52.85±1.01	123.75±1.43	46.85±1.07	48.27±0.58*
VLDL (mg/dl)	20.83±1.23	82.14±1.02	45.52±1.40**	52.21±1.50
TGL (mg/dl)	64.45±0.76	124.78±1.93	55.81±1.40	55.46±1.39

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

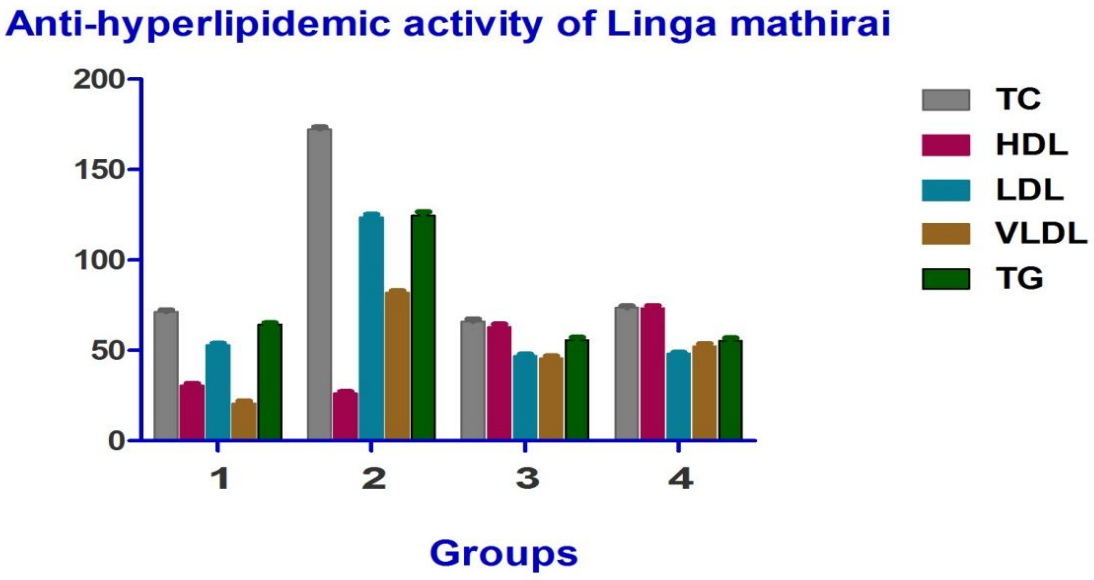


Chart 3:- Anti Dyspipidemic activity

DISCUSSION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, hypertriglyceridaemia and hypercholesterolaemia, resulting from defects in insulin secretion or action or both [67].

In diabetes mellitus along with hyperglycemia there is dyspipidemia always accompanied representing risk factor for coronary heart disease and other complications. Deficiency in insulin or insulin resistance may be responsible for dyspipidemia. Insulin has a inhibiting action on the enzymes involved in biosynthesis of cholesterol. Therefore deficiency of insulin results in the increase in level of LDL, VLDL, TG, cholesterol and decrease in HDL. Dyspipidemia produces further vascular complications and increases the severity of diabetes [68].

Anti-dyspipidemic activity of the test drug *Linga Mathirai* was conducted by Triton Wr 1339 induced dyspipidemia in animal model Wistar albino rats. The results were tabulated above.

The test drug *Linga Mathirai* of 200mg/kg b.wt showed significant changes in the lipid level. There is a significant decrease in the level of LDL, VLDL, TGL. There is a significant increase in the HDL also noted. The standard drug also showed the significant decrease in the LDL, VLDL, TG. But there is no significant increase in the HDL.

Hence this anti-dyspipidemic activity supports the anti-diabetic activity of the drug *Linga Mathirai* in giving an effective treatment of diabetes as a whole.

ANTI OXIDANT ACTIVITY

Table 24:- Effect On Oxidative Stress by *Linga Mathirai*

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5271	0.312	-	-
1.25	0.3873	0.278	26.21	40.89
2.50	0.3585	0.202	33.24	51.25
5	0.3365	0.084	60.10	74.07
10	0.2901	0.052	71.25	83.33
20	0.2611	0.034	76.57	89.62

***µg/ml: microgram per millilitre. Drug: Linga Mathirai (1.25-20µg/µl). Standard: Ascorbic acid(10mg/mlDMSO)*

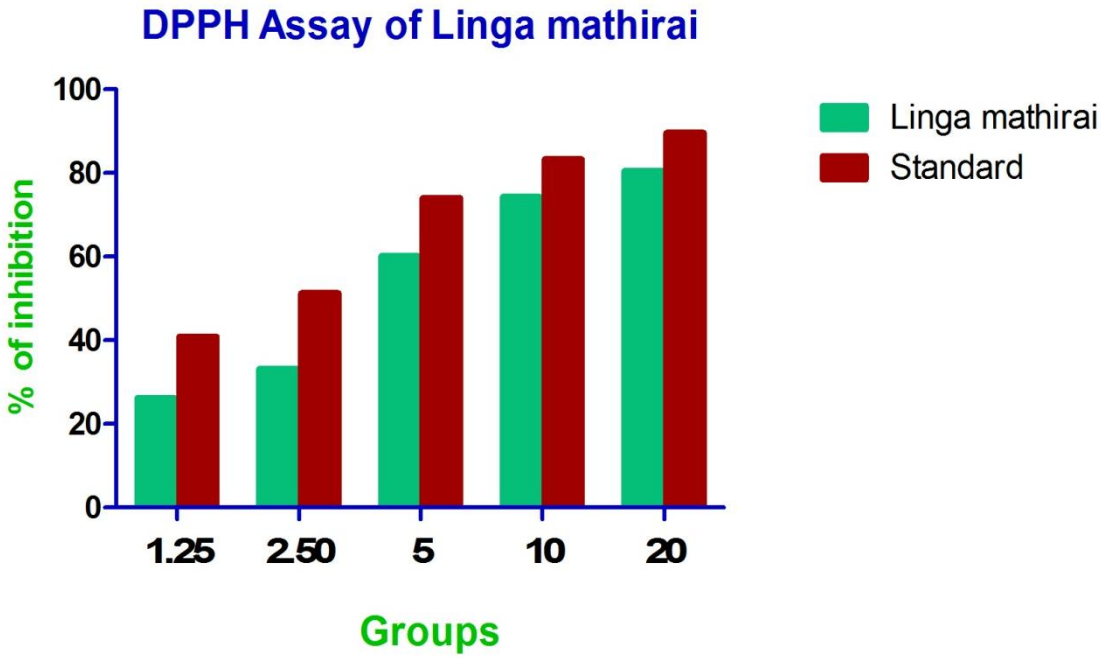


Chart 4:- Anti Oxidant activity

DISCUSSION

Anti-Oxidant study of the trial drug *Linga Mathirai* was conducted and the result has been given in the above table. The trial drug possesses significant Anti-Oxidant property.

Oxidative stress is one of the major pathophysiology of the disease. Oxidative stress is associated with the increased production of reactive oxygen species and impaired antioxidant defense systems, which cause lipid peroxidation, alteration in antioxidant enzymes and impaired glutathione metabolism [69].

In diabetes mellitus tissue damage is brought about mainly by the oxidative stress. There are certain enzymes involved in the Anti-Oxidant mechanism protecting the body from damage. The study shows the amount of the anti-oxidant enzymes increased significantly after the administration of the trial drug thus revealing its Anti-Oxidant property.

Hence the test drug *Linga Mathirai* possesses rich Anti-Oxidant property.

6. CONCLUSION

The drug *Linga Mathirai* was selected to validate the safety and its efficacy for diabetes in animal model (Wistar albino rats).

The ingredients of the drug was identified and authenticated by Gunapadam experts. The drug was prepared as per classical Siddha literary procedure and subjected to various studies to reveal its potency and efficacy of the drug.

The Organoleptic character and physico chemical studies were made to standardization of the drug *Linga Mathirai.* From the above studies, the *Linga Mathirai* is standardized as per AYUSH guidelines.

The analysis of biochemical, instrumental was made to know the presence of active ingredients in the drug which is responsible for its activity.

Here, the biochemical analysis showed the presence of potassium, by its synergistic effect, the drug as activity against the disease.

In instrumental analysis, FTIR showed the peak values represents the functional groups responsible for its activity. SEM picture explained the particle size of the drug. In ICP-OES described about the absence of heavy metals and its permissible limits which showed the safety of the drug.

Toxicity studies revealed about the acute and sub acute toxicity effect of the *Linga Mathirai* in the rat models. The drug showed no toxicity and mortality in both acute and sub acute toxicity. According to OECD guidelines, the haematological, biochemical parameters are investigated. There were no significant changes in the functional behaviour and in the normal values. Thus, it was greatly established the safety of the drug administrated for long time.

Pharmacological studies were done on the rat model for Anti diabetic, Anti-Dyslipidemic activity and In vitro study for Anti- oxidant activity.

In diabetes the main pathophysiology is increased oxidative stress which results in the tissue damage and it is the main reason for other complications. The anti-oxidant property of this drug is mainly due to the presence of phytochemicals

(flavonoids, phenols, etc.,) and other active ingredients which involve in scavenging the free radicals and prevents tissue damage and other complications.

In Anti diabetic activity, there was significant decreased blood glucose level and slightly increased body weight in the Streptozotocin induced Wistar albino rats.

In diabetes hyperglycemia is always accompanied with the dyslipidemia. Deficiency of insulin results in the increase in level of LDL, VLDL, TG, cholesterol and decrease in HDL. Dyslipidemia produces further vascular complications and increases the severity of diabetes. The drug showed significant decrease in the LDL and marked increase in the HDL than the standard drug.

In Anti dyslipidemic activity, showed significant decreased LDL, VLDL, TG, TC levels and marked increased HDL level in the triton WR-1339 induced dyslipidemic in Wistar albino rat models.

In Anti oxidant activity, there was significant effect of drug when compare to standard in DPPH assay.

Thus by surviving all the above factors, it is concluded that the drug *Linga Mathirai* is safe and potent drug against Anti diabetic and Anti-dyslipidemic activity with rich Anti oxidant activity. This will support the treatment and management of diabetes and its complication. In treating, the disease with this drug it has a synergistic effect of controls blood sugar level, lipid profile and also the oxidative stress brings a complete treatment of diabetes and its complication as a whole.

7. FUTURE SCOPE

The drug *Linga Mathirai* has its own potency in treating Diabetes mellitus in animal model which has been established in this study.

However, the mechanism of action by which *Linga Mathirai* produced its effect on decreasing the blood sugar level in experimental animals need to be evaluated in a scientific manner using specific experimental animal models and also multi-center clinical trials are required to understand the exact molecular mechanisms of action. So it could be used worldwide in treatment of Diabetes mellitus.

8. SUMMARY

- In the Siddha literature "***Skitcha rathina deepam***" the drug *Linga Mathirai* was selected for its Anti-diabetic, Anti-dyslipidemic and Anti-oxidant activities.
- In this dissertation, Introduction explained about the Siddha concept, about the disease diabetes in Siddha and modern aspect, prevalence of diabetes, mortality and morbidity rate and role of the drug in treating diabetes.
- The drug was prepared as per classical Siddha literature, the ingredients were identified and authenticated by *Gunapadam* experts.
- In review of literature, many studies were carried out about the drugs botanical aspect, *Gunapadam* aspect. Pharmaceutical review enclosed about the preparation of drug and the pharmacological review established the methodologies.
- The drug was subjected to various analysis such as physico chemical, biochemical and also instrumental analysis which provided the active ingredients present in the drug.
- Toxicological study was made according to OECD guidelines showed the study of the drug.
- Pharmacological studies were done to reveal the Anti-diabetic, Anti-dyslipidemic activity in animal models and Anti-oxidant activity of *Linga Mathirai* in DPPH assay.
- Results and discussion gave justifications to prove the potency of the drug.
- Conclusion explained the synergistic effect of all active ingredients and activities that supports the study.
- Thus, the herbo-mineral formulation *Linga Mathirai* is validated for its safety and efficacy for treating Diabetes mellitus, it would be a great drug of choice.

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